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# The Relation of Temperature and the Partial Pressure of Oxygen to Respiration and Growth in Germinating Wheat

BY
WARREN B. MACK

Submitted to the Board of University Studies of the Johns Hopkins University in conformity with the requirements for the Degree of Doctor of Philosophy

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# THE RELATION OF TEMPERATURE AND THE PARTIAL PRESSURE OF OXYGEN TO RESPIRATION AND GROWTH IN GERMINATING WHEAT*

WARREN B. MACK

(WITH SEVEN FIGURES)

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* Botanical contribution from the JOHNS HOPKINS UNIVERSITY, no. 107.

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## Introduction

GENERAL STATEMENT.—The present introductory discussion and the experimental study reported in this paper deal with the influence of temperature and of the concentration or partial pressure of oxygen in the surroundings, on the rate of evolution of $CO_2$ by higher plants in darkness and with adequate water supply. There is a considerable literature on the relation of higher plants to oxygen supply, and the literature on the influence of temperature and oxygen supply upon respiration in lower plant forms (such as bacteria, yeasts and fungi) likewise is extensive, but with the latter aspect of the subject we need not be concerned here.

Respiration rates may be ascertained in terms of the absorption of oxygen and of the disappearance of the respired plastic materials, such as carbohydrates, etc., but the rate of $CO_2$ production furnishes the most convenient and practical index of respiratory activity, and it is the index that has generally been employed in experimentation in this field; therefore measurements on $CO_2$ production will here be generally considered as indications of the respiration rate. The respiration rate is so dependent on temperature that no very extensive study of the process could be made without the inclusion of temperature among the measured variables and it is necessarily included here. The influence of light on respiratory activity appears to be slight in all cases where such influence seems to occur, excepting those instances where light operates through the photosynthesis of carbohydrate, in which instances it cannot be studied directly; indeed the experimental problem of the physiological control of respiration in green plants in light is a very special and difficult one. The water content of the respiring tissues is known to influence respiration very markedly, but only in its lower ranges; it may consequently be left out of account when the respiring material is well supplied with water. In a similar manner the influence of the partial pressure of $CO_2$ in the surroundings may be neglected when the gaseous products of respiration are allowed to escape freely so as not to accumulate within the cells to any significant extent. There are of course innumerable unessential substances that influence respiration, growth, etc., if present in suitable concentrations in the surroundings, their effects being sometimes to accelerate respiration and sometimes to retard it, according to their natures and their concentrations. Any of these substances may be made the subject of special study after the fundamental temperature-oxygen relations of respiration have been worked out for a given plant material. When such substances are absent or in sufficiently low concentrations it is not necessary to consider them.

After the study here reported had been completed another series of experiments was planned and carried out under similar conditions but in-

cluding a constant partial pressure of ethylene, an unessential substance that has recently assumed considerable importance in respect to the artificial treatment of fruits, vegetables, etc., for the market. The results of the ethylene experiments are reserved for another paper, although some aspects of the problem of ethylene influence are briefly considered in the present contribution.

The process of respiration is fundamental in all vital activity and increased knowledge concerning it is needed in many parts of the field of plant physiology and ecology, including agronomy, horticulture, forestry, etc. The influence of environmental conditions on respiration is inevitably involved whenever the more general processes of growth and development are being studied, as in discussions of seed germination [HUTCHINS, (**18**)], the behavior of roots in soils [CANNON, (**7**)], and the behavior of fruits, tubers, roots, cuttings, etc., in storage.

Because the respiration process as a whole is closely related to vitality in general and to the various kinds of growth and developmental changes that occur in a plant (such as enlargement, ripening, etc.), it is always desirable to give some attention to growth and development when respiration is to be studied. This is especially true when germination processes and ripening processes are considered, in which development and respiration are so interrelated as to be incapable of separation, even in theory and definition. Consequently the present paper includes some discussion of the relation of growth to oxygen supply and temperature.

A brief résumé of our knowledge concerning the influence of oxygen concentration and temperature upon the rate of evolution of $CO_2$ by higher plants in darkness and without water deficiency is presented below.

RELATION OF OXYGEN PRESSURE TO $CO_2$ PRODUCTION.—KOSTYTSCHEW, in his recent special monograph on plant respiration (**25**, p. 18–19) confines his review of the literature bearing directly on the influence of oxygen concentration to a single paragraph. His general conclusion is that, except for plants under extreme conditions, as when they are in nearly pure oxygen or in an atmosphere with less than 1 or 2 per cent. of oxygen, the concentration of that element has little effect on the rate of respiration. PALLADIN (**32**, p. 215) makes the brief statement that the partial pressure of oxygen in the surrounding atmosphere influences plant respiration without changing the value of the respiratory ratio. This statement is probably based on the results of GODLEWSKI'S work (**12**). KOSTYTSCHEW cites the work of DE SAUSSURE, WILSON, JOHANNSEN, and STICH, which seems to show that changes in external oxygen pressure, even to a considerable degree, effect little change in the rate of $CO_2$ production. But the results secured by GODLEWSKI point to a different conclusion.

In GODLEWSKI'S experiments (**12**) on radish seedlings a gradually diminishing concentration of oxygen was brought in an enclosed space, by the respiration of the seedlings contained therein, and the rate of $CO_2$ production steadily decreased as the partial pressure of oxygen was reduced. It might be thought, however, that this reduction in respiratory rate may perhaps have been related to accumulation of products of physiological activity as well as to the lowering of the oxygen pressure.

In WILSON'S experiments (**46**) sunflower seedlings gave off as much $CO_2$ when in a mixture of 1 volume of ordinary air and 4 volumes of hydrogen as they did when in ordinary air. When, however, the seedlings were changed from air to a mixture of 1 volume of air and 19 of hydrogen, the rate of $CO_2$ production fell from 18.6 mg. per hour to 12.1 mg. per hour, and when these same seedlings were returned to air the rate rose to 17.8 mg. per hour. The time of exposure was from 1 to 1.5 hours. In STICH'S experiments (**43**) on wheat seedlings and with an exposure time of one hour, a change in the concentration of oxygen in the surroundings from 20.8 per cent. (ordinary air) to 2.0 per cent. caused no change in the rate of $CO_2$ production. JOHANNSEN (**19**) found that an increase in the total pressure of ordinary air up to 2 and even to 5 atmospheres produced no effect on the carbon dioxide output of pea seedlings. Respiration of pea seedlings in nearly pure oxygen at ordinary pressures was no more rapid than in air; maize seedlings, however, produced $CO_2$ a little more rapidly when surrounded by nearly pure oxygen. When sunflower seedlings that had been in air were tested for 45 minutes in a mixture of 1 volume of air and 20 of hydrogen there was a decrease in the rate of evolution of $CO_2$ and when they were returned to air at the end of the 45-minute period the rate increased slightly, but the original rate in air was not regained in three-quarters of an hour after the return. The relative rates for air, air-hydrogen mixture, and air in succession were 14, 9, and 10, respectively.

It is interesting to note that WURMSER and JACQUOT (**47**) found a very striking dependence of the respiration rate of a kelp (*Laminaria saccharina*) on the oxygen content of the surrounding sea water. For oxygen contents of 5.2, 6.0, 12.0, 20.0, and 26.0 cc. per liter of water, the relative rates of $CO_2$ production were 1.0, 1.6, 2.4, 3.1, and 6.3, respectively, being approximately proportional to the oxygen content of the water. HEE and BONNET (**15**), however, in similar studies on higher plants, found only slight increases in the respiration of water-weed (Elodea sp.) and water milfoil (*Myriophyllum spicatum*) for increases in the oxygen content of the surrounding water from approximately 3 to 20 cc. of oxygen per liter. The time of observation for each oxygen concentration was from 3 to 4 hours, and the same individual plant was used throughout the series. In the field of

animal physiology, AMBERSON, MAYERSON and SCOTT (1) found that, for the lobster (*Homarus americanus*), the annelid worm (*Nereis virens*), the king crab, (*Limulus polyphemus*) and the blue crab (*Callinectes sapidus*), the rate of oxygen consumption was directly proportional to the oxygen tension of the sea water surrounding them, for a wide range of oxygen tensions. For a shrimp (*Palaemonetes vulgaris*) and a squid (*Loligo pealei*) the rates of oxygen consumption were directly proportional to oxygen tension of the sea water when this was below 50 per cent., and 30 per cent. of oxygen saturation, respectively. Above these values, however, the oxygen consumption of these two forms was found to be independent of the concentration of oxygen. NOMURA (31) found that oxygen consumption by a holothurian sea-slug (*Caudina chilensis*) was directly proportional to the oxygen tension of the surrounding water. These authors point out that oxygen consumption should be directly proportional to oxygen tension if there is an oxygen deficit in the tissues and if the rate of oxygen consumption is limited by the rate of diffusion of oxygen into the tissues.

Several investigators have found that in very small organisms in which diffusion of gases occurs very rapidly, oxygen consumption does not decrease appreciably with reduced oxygen concentration until a very low concentration has been reached. Below this critical pressure the oxygen concentration appears to limit the rate of functioning of the respiratory mechanism. SHOUP (41) has reviewed some of these studies and has shown experimentally that oxygen consumption by luminous bacteria is independent of oxygen pressure within the pressure range from 152 mm. of mercury (0.20 atm.) down to 22.8 mm. (0.03 atm.). Below the last mentioned pressure the curve for the rate of oxygen consumption as related to oxygen pressure is similar to curves for adsorption of gases at catalytic surfaces, which leads SHOUP to state that "luminous bacteria are so small that oxygen collecting at the catalytic surface of the oxidation mechanism of the cell becomes the limiting factor determining the rate of oxygen consumption rather than the oxygen diffusing into the cell." The respiratory activity of luminous bacteria ceased in pure nitrogen, and in pure oxygen the consumption of oxygen was irreversibly inhibited.

In the experiments on plant forms where no change in respiration was reported when the environmental oxygen pressure was increased or decreased, the periods of observation usually were short. JOHANNSEN'S measurements (19) of $CO_2$ production by sunflower seedlings successively exposed to air, air-hydrogen mixture and air, furnish evidence that the previous experience of the plants was an important condition in determining their respiration behavior in the short time periods employed. In the light of the studies of AMBERSON and his co-workers and of NOMURA, the

results secured by WURMSER and JACQUOT indicate that in the case of kelp, the rate of diffusion of oxygen into the tissues was a limiting factor in respiration on the supposition that the rate of diffusion would be directly proportional to the partial pressure of dissolved oxygen in the water. In the case of invertebrate animals differences in the responses of the several forms were probably determined by internal conditions of a regulatory nature.

If, under a given set of environmental conditions not dependent on oxygen supply, a plant or a plant tissue fails to show a changed rate of respiration corresponding to an alteration in the oxygen concentration of the surroundings, two possible explanations may be suggested. (1) The rate of oxygen supply within the cells may be proportional to the external partial pressure of oxygen but the rate of supply may not be a limiting condition under the circumstances; the lower rate of supply may be adequate, the higher rate producing neither increase nor decrease in respiratory activity. (2) An alteration of the partial pressure of oxygen in the environment may actually result in a change in respiration rate but there may be a marked time lag between the environmental change and the first appearance of the resulting internal effect, the observation interval being too short to show the response. The second suggestion emphasizes the extreme importance of duration in all physiological experimentation, a consideration that will be reverted to farther on.

INFLUENCE OF TEMPERATURE ON $CO_2$ PRODUCTION.—The literature concerning the influence of temperature on $CO_2$ production by plants was reviewed by KANITZ (**20**) in 1915, whose review included other life-processes of plants and animals, and by KOSTYTSCHEW (**25**, p. 16) in 1924. KANITZ attempted to analyze the relations of plant respiration and temperature in terms of temperature coefficients and pointed out that in many cases the quotient ($Q_{10}$) of the rate of $CO_2$ production for a given temperature divided by the corresponding rate for a temperature 10 degrees lower lies between 2 and 3 for some 10-degree ranges. The coefficient is lower, however, for 10-degree ranges in higher regions of the thermometer scale and higher for 10-degree ranges in lower regions of the scale. As FAWCETT has pointed out (**11**), the value of the 10-degree temperature coefficient for any process that has a temperature minimum and a temperature maximum must vary all the way from infinity (for 10-degree ranges partly below the minimum) to zero (for 10-degree ranges partly above the maximum), and it is not particularly important that the coefficient value is about 2 or 3 for some intermediate ranges. The interesting thing about temperature coefficients in general is not the actual value of the coefficient for any given 10-degree range but the manner of variation of the coefficient with regard

to the position of the 10-degree range in the temperature interval between minimum and maximum.

KOSTYTSCHEW emphasized the point that there appears to be no optimum temperature for the production of $CO_2$, above which the range is lower with higher temperature, for the rate gradually increases with rising temperature, to a maximum value at which it remains with still higher temperatures, until the thermal death point of the plant is reached. The same author referred also to the stimulating effect of abrupt temperature changes, as indicated by PALLADIN'S work on this subject (33). In PALLADIN'S studies, as cited by KOSTYTSCHEW, etiolated terminal buds of Windsor bean (*Vicia faba*) were kept on 10-per cent. sugar solution at low, medium and high temperatures for 3 days. When the rate of $CO_2$ production was then determined *at the medium temperature,* the rate was greater for the buds that had been kept at the high and at the low temperatures than for those which had been kept at the medium temperature and this had experienced no temperature change just prior to the measurement.

But KOSTYTSCHEW did not mention BLANC'S objections to PALLADIN'S interpretation. BLANC (5) pointed out that plant material kept for a time at one temperature is not to be considered as similar internally to material that has been kept for the same time at another temperature. In his own experiments, on etiolated terminal buds of Windsor bean (*Vicia faba*), young leaves of barley (*Secale cereale*) and embryos of ordinary bean (*Phaseolus vulgaris*), he ascertained the rate of $CO_2$ production for a given medium temperature, then exposed the material for an hour to a lower or higher temperature, and then again ascertained its rate of $CO_2$ production at the original medium temperature. The results showed a somewhat lower or somewhat higher rate in the second period at the medium temperature, according to whether the intermediate temperature was lower or higher, respectively, than the medium temperature. These observations were interpreted as failing to show stimulating effects for changes of temperature, although they indicate an after-effect of the intermediate exposure. It seems that the distinction among after-effects, lags, stimulations, etc., are very difficult and involved, and it may be best not to attempt any close analysis of the problem here suggested until much more experimentation has been carried out, with due attention given to all the influential conditions.

INFLUENCE OF $CO_2$ CONCENTRATION ON RESPIRATION.—SPOEHR and MCGEE (42) found that changes in the $CO_2$ content of the air surrounding a leaf had a temporary inverse effect on the rate of $CO_2$ emission. This effect varied in degree with different species. If the $CO_2$ concentration were maintained at a constant value after a change, the rate of $CO_2$ emission

finally became about the same as it was before the change. KIDD (**21**) found that both anaerobic and aerobic $CO_2$ production were reduced by $CO_2$ in the surrounding air. When oxygen was present, but in deficient amounts, so that some degree of anaerobic $CO_2$ production occurred, $CO_2$ in the atmosphere exerted no retarding effect. In ordinary aerobic respiration, with ample oxygen supply, however, the same quantitative relation was found between aerobic $CO_2$ production and the $CO_2$ concentration as between anaerobic respiration and $CO_2$ concentration. The inhibiting effect of $CO_2$ in the surroundings on germination of seeds, sprouting of tubers, and ripening of fruits, as found by KIDD and WEST (**22, 23, 24**), seems to be associated with its effects on respiration. The magnitude of the retarding influence was influenced by the concentration of oxygen in the surroundings.

INFLUENCE OF UNUSUAL CHEMICAL SUBSTANCES ON $CO_2$ PRODUCTION AND ON RIPENING PROCESSES.—Increased knowledge of the conditions that influence respiration promises to be valuable, and indeed quite essential, to an understanding of many forms of chemical stimulation, as, for example, the influence of ethylene on the ripening, blanching, etc., of fruits and vegetables, which has recently attracted much attention. The conflicting nature of the results reported by those who have made careful studies on the influence of ethylene suggests that this practical application can become understood and standardized only in terms of the basic physiological processes and rate changes that are involved, notably the phenomena of respiration. Some of the methods employed in treating plant material with this gas surely produce changes in the partial pressure of oxygen and of $CO_2$ about the stimulated tissues; for treatment in many instances has consisted in enclosing in a tight container the material to be treated, without any continuous flow of the gas mixture through the confined space. The influence of ethylene, or of any other gas, cannot be satisfactorily studied unless the method of continuous gas flow is employed. With suitable continuous flow of a known gas mixture the partial pressure of the gases used may be maintained almost constant and $CO_2$, or other gases produced, may be constantly removed, avoiding troublesome accumulation. An experimental study on the influence of ethylene upon respiration and growth of young wheat seedlings was carried out in connection with the investigation reported in this paper, but the results obtained, together with some discussion of the general problem of ethylene influence, are reserved for another publication, as has been noted.

INFLUENCE OF OXYGEN PRESSURE ON GROWTH AND DEVELOPMENT.—Among the early investigators who studied the relation of oxygen to growth and development should be mentioned the Swedish chemist, SCHEELE (**38**) (who

discovered oxygen independently in 1771), HUMBOLDT (17), ROLLO (36), DE SAUSSURE (37) and DÖBEREINER (10). Their experiments dealt mostly with germination of seeds and growth of seedlings in nearly pure oxygen and in ordinary air. Germination was more rapid in oxygen than in ordinary air, but seedling growth was less vigorous in oxygen. DÖBEREINER studied the enlargement of seedlings in air with increased and with reduced total gas pressure. Barley plantlets grown in air with half the ordinary total atmospheric pressure were more spreading, softer, and somewhat shorter than others grown at the same time in air with a total pressure of 2 atmospheres. There was more guttation in the plantlets grown with low pressure and this was considered to indicate higher turgidity. The differences in growth were attributed to turgor differences.

This subject was studied more thoroughly in the quarter century between 1873 and 1900, by BÖHM, BERT, WIELER, JENTYS, JACCARD and SCHAIBLE. BÖHM (6) found that the growth of plants at the expense of reserve materials was slower in nearly pure oxygen at a pressure of 1 atmosphere than it was in ordinary air, but growth in oxygen was more rapid (and like that occurring in ordinary air) if the oxygen tension were reduced until it was equal to that of the oxygen in ordinary air, the reduction being accomplished either by reducing the total gas pressure or by diluting the oxygen with hydrogen. BERT (3) concluded that variations in either direction from the ordinary partial pressure of oxygen as it occurs in the air resulted in decreased growth, and he attributed these differences in growth rate to differences in oxygen tension.

These studies of BERT, WIELER, JENTYS and SCHAIBLE were summarized by PFEFFER (35), who came to the generalization that most aerobic plants fail to grow in air at ordinary atmospheric pressure when the oxygen content of the air is less than from about 0.1 to about 3 per cent. by volume. He interpreted the effects of considerable differences in the total pressure of the surrounding air as influences on turgor, thinking that suitably diminished pressure increased turgor and so tended to accelerate enlargement, while sufficiently increased pressure at first reduced turgor and tended to reduce enlargement, though later it might evoke apparently adaptive responses of an opposite character, which PFEFFER designated as counter-effects. As to the influence of partial pressure or percentage of oxygen in the air about the plant, PFEFFER concluded that sufficiently increased oxygen percentage in air, with the ordinary total pressure, generally retarded growth, an effect apparently due to the oxygen itself, acting as a chemical influence. He pointed out that the cardinal points (minimum, optimum, maximum) of partial oxygen pressure, are dependent upon other influential conditions, especially upon the internal ones that are related to phase or stage of development, previous experience of the plant, etc.

In none of the experiments considered above were nearly all of the important influential variables taken into account. With green plants in the presence of effective light and with adequate supply of $CO_2$ the partial pressure of oxygen may be supposed to be high, at least in the illuminated green parts, this partial pressure being largely independent of the oxygen conditions of the surrounding air. The partial pressure of oxygen must be much lower in green parts after a period in darkness, and generally lower than that of the surrounding air in any parts into which oxygen does not diffuse at an adequate rate, as in the deeper-lying tissues of succulent leaves, stems, fruits, etc. On the other hand, when the aerial parts of green plants are kept in darkness or in sufficiently weak light for long periods the phenomena of etiolation are likely to lead to great complications in any attempted analysis of the relations between environmental conditions and the rate of enlargement.

HEUMANN's more recent studies (**16**) did not greatly add to our information on the direct action of oxygen with respect to growth, for the reasons just suggested. His conclusion is that the acceleration of growth brought about by reduced partial pressure of oxygen in the air is caused by oxygen-hunger in the plant. He supported this idea with the observation that the morphological response to low oxygen supply was an increased area of organs, such as leaves, through which the oxygen of the surrounding air penetrates to the plant interior. The effects observed are thus attributed to an oxygen influence on general metabolism rather than to a direct influence on growth itself.

HARRINGTON (**14**) noted that when wheat seedlings were in an atmosphere containing 36 per cent. of oxygen growth was nearly twice as rapid as when they were in ordinary air, while further increases in the partial pressure of oxygen resulted in slower growth. He did not mention that light was excluded but light influence through photosynthesis may be supposed to have been of small consequence in these experiments, since seedlings in the early stages of their growth are not dependent to any considerable degree on the products of their own photosynthetic process, which is not rapid in these early stages.

It is evident that the relations between oxygen supply and growth in higher plants are very difficult to evaluate. Although the problem of growth-oxygen relations may be simplified to some extent by the exclusion of light in the experiments, yet the complications arising from the occurrence of etiolation cannot be avoided if the tests are continued throughout extended periods of time. The special responses of etiolation may be rendered less important if short experiment periods are employed, or the experimentation may be confined to germinating seeds or other tissues and organs

that may be kept for long periods in darkness without special effects arising from the absence of light. In any event, the influence of oxygen supply or of the partial pressure of oxygen in the surroundings must be studied with reference to as many of the vital processes as possible if we hope to approach this general problem successfully. For first approximations the general metabolic processes may perhaps be considered as summed up in terms of the rate of respiratory output of $CO_2$, and growth may be superficially estimated in terms of enlargement and other easily observed features of morphological development. Whenever the time period of an experiment in this field is sufficiently long observations should surely be made on both growth and respiration if such observations are at all possible.

The time relation of respiration and growth processes.—The importance of the time factor in physiological experimentation and discussion is suggested by some of the considerations in the foregoing paragraphs. Whenever the influence of conditions determining the rate of a process is to be analyzed it is essential that time be taken into account not only with reference to the measurement of the process rates themselves but also in the quantitative measurements of the influential conditions. Some aspects of this principle have been emphasized by Lehenbauer (**26**), Fawcett (**11**), Haasis (**13**) and others and it is coming to be generally appreciated. Its special bearing in the present study lies mainly in the fact that developing organisms usually alter as they develop, respiration and growth rates increasing or decreasing with the progress of development. The only way in which a rising or falling process rate may be studied is by means of several short test intervals each of which is so chosen as to represent a suitable portion of the longer period during which the acceleration or retardation occurs. If test periods are too long the corresponding mean rates may be misleading, which is also true if total experiment periods are too short. When a rate is found to be altering with time, under maintained external experimental conditions, it is desirable to employ conveniently short, generally successive, test periods and to continue the experiment either till the rate becomes relatively constant, till it passes through a critical stage at which the sign of its acceleration changes, or till its acceleration becomes constant. When a new set of external conditions is brought to bear the behavior of the organism is usually related for a while to both the old set of conditions and the new set and the only way by which such lagging influences may be studied is through the employment of sufficiently short observation intervals in the earlier portion of the experiment period.

The length of the whole experiment period and the lengths of the partial periods employed for the rate tests are surely very important in such experiments as are required in the field here considered, for the rate of $CO_2$ evolu-

tion from germinating seeds and young seedlings increases with time, even when all external influences are maintained. These time relations are most important when it is necessary to alter the external conditional complex at the beginning of an experiment, as when seedlings are produced under one set of environmental conditions and are then transferred to another set for an experiment.

The last statement implies a consideration that is sometimes expressed by saying that the behavior of a given organism is partly determined by its past experience and partly by its current environment. Past experience, whatever it may have been, has resulted in the present internal conditions of health, vigor, tone, etc., and present behavior must really be considered as wholly controlled by the combination of current internal and current external influences. But we may be allowed to use the expression past experience to imply present internal characteristics as these have been molded by the environment and the organism operating together in the past.

In experiments that involve a change in the environmental complex, whether from preliminary treatment to the experimental conditions or from one set of conditions to another in the course of the experiment, the resulting changes in physiological processes are usually not abrupt and occur more or less gradually after the change in environment. It is therefore very difficult, as has been said, to separate the results of current influences from those of past influences. The immediate need for this sort of analysis may be avoided if we can find means for adequately defining or describing our experimental material at the beginning of an experiment, being as sure as may be that all coordinate experiments start with material having the internal characteristics thus described.

The estimation of the relative importance of past experience, current environment, and apparently adaptive responses can be arrived at most readily by analysis of the progressive changes in process rates which occur after the lapse of different lengths of time subsequent to specific changes in the environmental complex. As an illustration, let the plant material be changed from one environment to a second, temperature and the partial pressure of oxygen being considerably lower in the second than in the first. Subsequent observations might show the effects of several kinds of response as indicated by the respiration rate: The rate might decrease because of the lower temperature or because of the lower oxygen pressure or because of both these changes acting together; the rate might increase because of one or both of the changes and a tendency toward increase or decrease might be masked by a more pronounced tendency in the opposite direction; a tendency toward gradual increase would generally be expected, because the plant material continues to develop; and various apparently adaptive re-

sponses or counter effects might influence the rate as time went on. It is evident, therefore, that the acceleration of the rate of any process would only rarely be constant, and that changes in acceleration occurring under different sets of conditions, and in different test intervals are valuable clues to the separate responses that really occur.

## Experimental methods

General plan of the experiments.—The experiments described in this paper were carried out in the Laboratory of Plant Physiology of the Johns Hopkins University, between October, 1928, and March, 1929. The writer greatly appreciates the facilities placed at his disposal by the Johns Hopkins University and also the leave of absence granted to him by the Pennsylvania State College, for the academic year 1928–29, through which these studies were made possible. The writer is glad to express also his personal gratitude to Dr. Burton E. Livingston, director of the Laboratory of Plant Physiology of the Johns Hopkins University, for sincere interest, for valuable suggestions in the planning of the experiments, and for kindly criticisms on the preparation of this report.

These experiments were designed to take into consideration the difficulties brought out in the preceding introductory discussion. The process rates studied ($CO_2$ production and growth) were considered as the resultants of the combined action of the internal and environmental complexes of influential conditions. The influential conditions were considered as of two categories: (a) the background or parameter conditions, which were not consciously varied from experiment to experiment, and (b) the experimental variables (temperature and partial pressure of oxygen) which were maintained constant throughout a given experiment or any repetition of it, but differed from one experiment to another. The background conditions included (1) the internal conditions of the seedlings, which were as nearly alike as possible at the beginning for all experiments, being determined by the nature of the seeds and the preliminary treatment (germination procedure) to which they had been subjected; and (2) all external influential conditions excepting the two experimental variables. The background environmental conditions were planned to be alike for all experiments, being maintained nearly constant throughout the period of each experiment. These comprised water supply, salt supply, and light, the last having a value of zero because the cultures were kept always in darkness except for occasional momentary exposures to weak diffuse light for observations. Other influences which were kept constant or nearly so were the length of the experiment period and the lengths of the successive observation or test intervals within this period. For the different consecutive test intervals, how-

ever, the time was really an experimental variable; for example, the seedlings were of course younger in the first test interval than in the second, and the respiration rates in these intervals therefore corresponded to seedlings of different ages and different stages of development.

The main features of the general plan may be summarized as follows. Seeds of a specified stock were germinated by a specified technique and the resulting seedlings were allowed to develop in cultures, for a definite experiment period (46 hours) following a short period for transfer (0.5 hour) and adjustment (1.5 hour). Each experiment consisted of a single culture of 100 seedlings submerged in 250 cc. of standard nutrient solution, exposed to a certain maintained temperature, with a certain partial pressure of oxygen in the oxygen-nitrogen mixture continuously passing through the solution in the culture flask. Five experiments were carried out at the same time, constituting an experiment series. All of the five cultures in any series had the same oxygen-nitrogen mixture, but each differed from the other four with respect to its maintained temperature. Thus the oxygen-nitrogen mixture was the same for all experiments of the same series or repetitions thereof but differed for different series, while any given maintained temperature was employed for only one of the five experiments in any series. Each experiment thus differed from the others with respect to its combination of maintained temperature and oxygen pressure. Beginning at the end of the adjustment period (1.5 hour) the rate of $CO_2$ production was ascertained for five consecutive test intervals of 4, 6, 12, 12, and 12 hours, respectively, and observations on seedling development were made at the ends of the intervals, especially at the end of the fifth interval, which terminated the experiment in each case. The results show hourly respiration rates (as indicated by $CO_2$ production) for the several consecutive test intervals of each experiment and also notes on the corresponding growth rates for each experiment and each set of results corresponds to the specified oxygen-temperature complex that prevailed in the experiment from which they were derived, acting in connection with the internal and external background conditions previously mentioned.

The wheat seedlings.—The wheat seed used in these experiments was of the Nittany variety, a pure-line winter wheat selected by Dr. C. F. Noll, of the Pennsylvania Agricultural Experiment Station. The stock used was of the 1928 crop and was secured from the originator. It was stored in an aerated container at a temperature of about 20°–25°. The experimental lots, selected to include only sound, well-filled grains, showed a germination percentage of about 98 per cent., under the germination conditions provided. Twice as many seeds were germinated for each series of experiments as were required for the five cultures in the series, in order to allow for selection of

seedlings within relatively narrow limits. Thus 1,000 seeds were used for each experiment series, and five cultures of 100 seedlings each were regularly set up at the same time.

The method of germination, which was adopted after some preliminary tests, consisted in placing the thousand selected seeds in 500 cc. of distilled water in a 600-cc. Pyrex Erlenmeyer flask, and aspirating ordinary air through the water rapidly enough to cause a little movement of the seeds throughout the germination period, during which the flask was kept in the 20-degree compartment of the constant temperature apparatus. Temperature, moisture supply, and aeration were thus very nearly constant throughout the germination period and were practically the same for successive lots of seeds. Using the same quantity of water and the same temperature for each lot of 1,000 seeds gave assurance that changes brought about in the water by the soaking and germinating seeds (such as the outward diffusion of materials, which conceivably might influence germination) were of the same kind and occurred at about the same rates in all cases. No attempt was made to control the chemical content of the air aspirated through the water about the seeds; all the work was done in a well ventilated greenhouse in which plants were growing and no poisonous or stimulating gases could have been present in the air in significant amounts.

The length of the germination period was 42 hours, generally with a deviation of not more than a few minutes. In no case was the deviation more than 40 minutes. The temperature chosen for germination, actually about 19.5°, which is near the optimum for germination of wheat, lies at the middle of the range of the maintained temperatures used in the experiments. The mean temperature of the 20-degree chamber was a little below 20°, for there was a slight cooling at night. The successive lots of seedlings were as nearly alike as could be ascertained by ordinary observation, but of course there was considerable observable variability within each lot of 1,000 seedlings; in spite of the preliminary selection the seeds of a lot did not all reach exactly the same stage of germination in the 42-hour period. Nevertheless, the high degree of uniformity of the seeds and the uniformity of the germination treatment made it possible in every case to select the requisite five lots of 100 seedlings each, so that the populations of all cultures throughout the study, whether of the same or of different series, were apparently alike. The limits of development between which seedlings were selected were such that the coleoptile was always exposed, and the rootlets had not yet broken thorugh the coleorhiza.

It was of course conceivable that the lots of seed used for the later series of experiments might have differed constitutionally in some respects from the lots used for the earlier series, for the seed of the later lots was about

4 months older than that of the earlier ones and significant internal changes might possibly have occurred in the storage period. Some of the earlier experiments were repeated near the close of the work and the results of these showed clearly that the stock of seed had not altered significantly, as will be shown farther on when the experimental data are presented.

The standard conditions for germination constituted the environmental complex for the first 42 hours of soaking and germination, from which the subsequent experimental complex differed with respect to temperature, oxygen concentration and salt environment. Only in those particular experiments in which the maintained temperature was 20° and the partial pressure of oxygen was 20 per cent., was the oxygen-temperature complex the same for the experiment period as for the germination. In all other experiments, the seedlings experienced an abrupt lowering or raising either of oxygen pressure or temperature or both, at the beginning of the experiment. The $CO^2$ pressure in the environment was nearly constant throughout both the germination and experiment periods, $CO_2$ being removed by the constantly flowing gas stream in both cases, about as rapidly as it was formed. Since the seeds were germinated in distilled water and were in a standard weak nutrient solution for the experiments, all seedlings experienced a change in salt concentration and osmotic relations at the time the experiments were set up. This change, however, was the same for all experiments. The subsequent physiological activities of the seedlings through absorption and excretion, surely altered the solution during the progress of an experiment and the kind and degree of alteration may have been different for different experiments. Some of the experimental data indicate that these sources of discrepancy had no significant effect on the rate of $CO_2$ production, from the standpoint of this study. Nevertheless it would have been theoretically better if the same standard nutrient solution had been used for germination as for the subsequent experimental cultures, and if a constant flow of nutrient solution had been arranged as well as of the gas mixture. (On the theoretical advantages of continuously flowing solutions for solution cultures see Trelease (**45**) and Shive (**40**). No technique has been developed, however, for maintaining a constant flow both of gas and of solution through a culture from which $CO_2$ is to be removed and measured as it is produced.

The maintained temperatures used and their control.—The maintained temperatures tested were 10°, 15°, 20°, 25°, and 30° C. Preliminary tests showed that the experiment period and its consecutive observation intervals would need to be inconveniently long for temperatures below 10°, because of the slowness of growth and respiration at such low temperatures, especially for the earlier observation intervals. Consequently no temperatures below 10° were included in this study. Cultures at 30° showed but

little growth and there were some appearances of breakdown in seedlings held at that temperature two days or longer, while disintegration appeared in about 36 hours with a temperature of 40° and no growth was evident at that temperature. Since this study was not to deal with lethal and *post-mortem* phenomena, the range of temperatures tested did not extend beyond 30°. Temperature differences of 5 degrees were used partly because of convenience and partly because they were about as small as were warranted by the magnitudes of the temperature influences that were observed.

The battery of temperature chambers at the Hopkins Laboratory of Plant Physiology [Livingston and Fawcett (**28**), Haasis (**13**)] consists of a linear series of seven chambers with water jackets, separated from one another by uninsulated sheet-metal partitions, but insulated above, below and at the sides. The top of the apparatus is formed by the insulated wooden covers of the water baths. These are removable, but for convenience in manipulation, each is provided with three ½-inch holes, through which tubes leading to cultures, etc., may pass, or thermometers may be inserted, and also with a rectangular opening (23 x 28 cm.) closed by means of a removable insulated lid. Heat is supplied at one end of the series from a thermostatically controlled hot tank and removed at the other end by means of a cold tank thermostatically controlled by a mechanical refrigeration machine. Each bath is furnished with a mechanical stirrer, which slowly rotates in the water jacket around the cylindrical chamber, and all parts of each chamber are at practically the same temperature, but the temperature of each chamber differs from that of the adjacent chamber or chambers to a degree determined by the settings of the two thermostats. The temperature of the greenhouse in which the series of chambers stands was subject to fluctuations as great as 15° within a period of twelve hours or less and such changes in the temperature of the external air influenced the chamber temperature to some extent, in spite of the insulation, especially that of the chambers near the middle of the series, where the daily fluctuation, though ordinarily less than 1.0° was occasionally as great as 2.0°. The temperature of the middle chamber (20°) was usually from 0.5 to 1.0° too low for about 8 hours each night, and the average temperature of that chamber was really about 19.5°. Only five chambers were used in this study, the two end ones of the series of seven being idle. The chambers used were operated to give maintained temperatures close to 10°, 15°, 20°, 25° and 30°, as has been indicated.

Temperature records were made in the 10-, 20-, and 30-degree chambers, by means of small Richard thermographs, which were kept there throughout the experiments. The temperatures of the other two chambers were estimated by interpolation, with occasional direct observations by means of ordinary thermometers in these chambers.

THE GAS CONTROL.—The desired partial pressures of oxygen were secured by mixing compressed commercial oxygen and commercial nitrogen in a gas cylinder such as commercial gases are supplied in, the capacity of the cylinder being well above 2,000 pounds per square inch. A given mixture was prepared by connecting charged cylinders of nitrogen and oxygen successively to a previously discharged cylinder, by means of a copper tube (about 75 cm. long) provided with a suitable coupling at either end and with an ordinary pressure gauge closing a T-outlet in the middle. Each gas was allowed to flow into the mixing cylinder until the gauge gave the required reading, the proportions being measured in terms of pressure. A mixture of 10 volumes of oxygen and 90 volumes of nitrogen was secured, for example, as follows: Oxygen was first allowed to enter the mixing cylinder till the gauge showed a pressure of 100 lbs., after which the valve of the mixing cylinder was closed and the oxygen supply tank was removed. Then nitrogen was allowed to flow into the mixing cylinder till the gauge showed a pressure of 1,000 lbs., after which the valve was again closed and the connecting tube was removed. The result was a tank of gas mixture of practically the required proportions and with a pressure of 1,000 lbs. per square inch. A tank of gas mixture might be kept closed till needed, when it was connected to the gas line leading to the culture flasks.

The compositions of the commercial gases and of the mixtures containing less than 30 per cent. of oxygen were ascertained through absorption of the oxygen by phosphorus. In the analysis of commercial oxygen a small amount of the gas was introduced into the eudiometer and its volume was measured, after which commercial nitrogen (from which the oxygen had previously been absorbed by means of phosphorus) was added in quantity sufficient to reduce the percentage of oxygen to less than 50 per cent. The mixture thus formed was then analyzed and the necessary computations were made.

The gas mixtures were delivered into the tubing lines leading to the culture flasks at a pressure regulated by means of a Hoke Phoenix Regulator or pressure-reducing valve. The maintained rate of flow through a flask was determined by the pressure of the supply and by an orifice control in each tube. Before reaching the separate lines leading to the cultures, the gas was passed first through a tube (of 18-mm. bore and 30 cm. long) containing soda lime, to remove any $CO_2$ which might be present, and then through concentrated $H_2SO_4$, to remove moisture, which might have tended to clog the orifice controls. The gas was then delivered to the separate lines through a 5-branched manifold of copper tubing.

The orifice controls were similar in principle to those described by HUTCHINS (**18**). Each consisted of a section of glass tube (of 5-mm. bore

and 12 cm. long) with a slight constriction about 3 cm. from one end and a plug of alternate layers of dry cotton and powdered chalk packed in the longer portion, against the constriction. The gas passed through the plug before reaching the constriction and the pressure of the gas thus tended to hold the plugs firmly in position. Alternate layers of cotton and chalk were found to be more easily adjusted than a single mass of kaolin or chalk between two cotton plugs, which was employed by HUTCHINS. The controls were adjusted so that the gas flow was 3 cc. per minute for each pound of pressure registered on the gauge of the pressure-reducing valve. The rate of gas flow (measured by means of a eudiometer) was found to be directly proportional to the pressure with this arrangement and it could therefore be controlled at will by setting the valve for the proper delivery pressure. The orifice controls required one or two slight adjustments in the first few days of use, perhaps because of the drying of the cotton or chalk. After these preliminary adjustments, the standard ratio of flow rate to gauge reading remained constant as long as the controls were in use, as was shown by occasional tests.

Between the orifice control and culture flask the gas was led through a telltale, which consisted of a rubber-stoppered bottle with inlet and outlet tubes arranged so that the gas bubbled through about 50 cc. of very dilute barium hydroxide solution colored with a few drops of phenolphthalein. Each telltale stood alongside the culture flask it served, in the same temperature chamber, and the incoming gas was here brought to the temperature of the culture chamber before entering the flask. The constant pink color of the solution indicated the absence of $CO_2$ in the gas stream. Also, moisture was added to the gas here, so that the volume of the nutrient solution in the culture flasks was not significantly altered by evaporation or condensation.

The total gas pressure above the solution in the culture flasks was always slightly higher than the prevailing atmospheric pressure, because of the hydrostatic pressure of the solution in the absorption-tower and in the telltales, but this difference was considered as negligible, especially since the pressures were the same for all cultures in a given series of experiments. The partial pressure of $CO_2$ in the culture flasks was kept practically at zero, by sweeping out the $CO_2$ as rapidly as it was formed.

The rate of gas flow through the culture flasks was established at 20 cc. a minute, after a number of preliminary experiments on the effect of variation in the rate of gas flow. In these preliminary experiments a gas mixture high in oxygen was used, and the rate of flow was different for successive experiment series, with differences of 5 cc. or less for a range from 5 cc. a minute to 20 cc. The gas mixture was presumed to be nearly pure oxygen,

but was not analyzed. It was subsequently indicated, however, to contain about 85 per cent. of oxygen by comparison of results with it and with mixtures the composition of which was known. Experiments were performed also with a mixture containing approximately 20 per cent. of oxygen, some with a rate of gas flow of 5 cc. a minute and some with a flow of 20 cc. a minute. In the experiments with a high percentage of oxygen, the rate of $CO_2$ production was greater for increased rates of flow up to 15 cc. a minute, but showed no significant changes for higher rates. Growth at the higher temperatures also increased as the rate of gas flow was raised, up to 15 cc. a minute, but did not change for rates greater than this. The proportional increase in $CO_2$ production for an increase in rate of gas flow from 5 to 20 cc. a minute was found to be practically the same for seedlings in 20 per cent. of oxygen as it was for seedlings in the high percentage of oxygen. This increase in $CO_2$ production for greater rates of gas flow was probably due to more thorough mixing of the nutrient solution, which resulted in a more uniform supply of oxygen to all the seedlings and more rapid removal of $CO_2$ from them. The lowest rate of gas flow supplied oxygen to the culture at a rate far in excess of oxygen consumption by the seedlings, as indicated by their $CO_2$ output. The higher rate of gas flow resulted in fairly rapid convection throughout the nutrient solution; a droplet of India ink became evenly dispersed throughout the solution in less than a minute when the gas flow was 20 cc. a minute.

The rate of gas flow therefore was established at 20 cc. a minute, since this rate was evidently more than sufficient to maintain $CO_2$ production and growth at maximum rates, as far as oxygen supply was concerned, for all the temperature-oxygen combinations tested.

The culture flasks.—The culture flasks were of Pyrex glass, of the Erlenmeyer form, with capacity of 300 cc. Two sets of five were used, so that one set could be cleaned and prepared for an experiment while the other five were still in use. Each flask had a tightly fitting 2-hole rubber stopper through which the inlet and outlet tubes were led. Because flexibility was desirable these tubes were of lead, but the metal did not come in contact with the culture solution at any time. The lead portion extended only about 15 mm. below the stopper, and the inlet tube was extended by a glass portion, attached by an ordinary rubber-tubing coupling, nearly to the bottom of the flask. The entering gas mixture bubbled through the culture solution, and passed out through the other tube. The outlet tube led directly to its own absorption apparatus.

To avoid any possibility of leakage the stoppers of the culture flasks (and also the stoppers of the telltale bottles) were secured by means of special clamps. A horizontal triangle of $\frac{1}{8}$-inch brass plate with an opening in its

center (to accommodate the inlet and outlet tubes) rested on the top of the rubber stopper and was connected with a base piece by means of three vertical 3/16-inch brass rods threaded at either end and provided with brass nuts (from discarded electric dry cells). The base piece on which the flask stood was a horizontal hexagon (about 2.5 cm. thick) of 2-ply wood with three holes near its edge, for the vertical rods. When the clamp was in place and the nuts were tightened the two plates were drawn toward each other and the stopper was held firmly in position in the neck of the flask.

Tubing and connections.—Most of the tubing lines were of glass, with wired couplings of thick-walled rubber tubing, the joined ends being in contact. Where flexibility was required lead tubing was employed, it being joined to the glass sections by wired couplings of rubber. There were no joints between the orifice control and the absorption apparatus excepting those formed by the rubber stoppers of the culture flask and the entrance telltale, which were very tight. All lead tubing used was first tested for pin-holes, etc., by applying a gas pressure of 20 pounds per square inch with the tubing under water. The tubes for liquid were of glass or flexible rubber.

The absorption apparatus.—The absorption apparatus embodied some of the principles of an apparatus described by Carrick (8), with modifications to allow titrations to be made within the apparatus. The details of construction are shown by the diagram of figure 1. The essential features are: the absorption tower, A; the standard acid burette, B; the titration bottle, G; the vent, E, guarded with the soda-lime tube D; the siphon outlet, I; the inlet for standard alkali solution (or water for washing), J; the outlet telltale, M; an additional vent, K, and the outlet, L, both guarded with soda-lime tubes; and the gas inlet tube, F, connecting the apparatus with the culture flask, from which it is the outlet tube. A complete absorption apparatus was provided for each of the five culture flasks, being permanently mounted on a shelf at the side of the constant temperature chamber in which the culture flask was located.

The absorption tower (A, fig. 1) consisted of a heavy-walled glass tube (18 mm. in inside diameter and 55 cm. long) containing a column of glass beads (3–5 mm. in diameter) about 35 cm. high, the beads resting on an inverted glass vial (C) about 7 cm. high, with 5 or 6 holes blown in its bottom. Both vial and beads were found to be required for complete absorption of the $CO_2$ brought from the culture flask in the gas stream. The inverted vial provided a reservoir of absorbing solution, into which the entering gas bubbles were discharged and in which they were held for a short period, until succeeding bubbles caused the trapped gas to move upward through the holes and among the beads.

The outlet telltale (M, fig. 1) was simply a test tube (20 x 150 mm.) provided with a 2-hole rubber stopper and inlet and outlet tubes, so arranged that the gas escaping from the absorption tower bubbled through a measured quantity of standard barium hydroxide solution before being discharged. The outlet of the telltale was of course guarded by means of a soda-lime tube. Absence of any white precipitate, in this telltale, which was in plain sight, made it certain that no $CO_2$ had found its way through the absorbing tower.

When absorption of $CO_2$ was in progress, all of the three rubber connections marked X on the diagram (fig. 1) were closed with Hoffman clamps. A definite quantity of standard barium hydroxide solution (0.2 normal) was measured from a charging burette into the absorption tower through the solution inlet (J, fig. 1) and was washed down with a small quantity of $CO_2$-free water. The pressure of the gas entering through the gas inlet (F, fig. 1) held the alkali solution in the tower and the gas bubbles rose through this solution, being delayed and broken up by the vial (C) and the glass beads. Thence the gas stream was conducted through the outlet telltale (M) and was finally discharged through the discharge outlet (L).

When a determination was to be made, at the end of a test interval, the vent (K) at the top of the absorption tower was opened first. A few drops of phenolphthalein solution was introduced through the solution inlet (J) and a tube leading from a reservoir of distilled water above the apparatus was then connected to the solution inlet. The vent (E) on the titration bottle was then opened and the solution in the tower was drained into the titration bottle (G) through the tube H. Water was then allowed to flow in through the solution inlet (J) until all traces of pink color had been washed down. The excess of alkali was then determined by titration with standard HCl solution (0.1 normal), from the burette B. The connections were sufficiently flexible to allow shaking of the titration bottle (G), during the titration. When the end-point was reached the neutralized solution was allowed to flow out through the discharge siphon (I), by opening the clamp IX. The siphon tube itself was not emptied, since the water in it provided an insurance against leakage past the clamp. Tower and bottle were finally washed with distilled water, and a new charge of standard alkali solution was introduced as at the beginning, all clamps being properly adjusted.

The standard acid solution.—The standard acid solution used for titration and for standardizing the standard alkali solution was approximately 0.1 normal (0.1132 N) HCl. This was standardized originally against dry sodium carbonate, with methyl orange as indicator, and the standardization was repeated from time to time during the progress of the experimental work, to ascertain whether changes occurred in the concentra-

tion of the acid under the conditions of storage. Eighteen liters of the standard acid solution were prepared at the beginning of the work, and this was sufficient for the entire series of experiments. This stock was kept in an 18-liter bottle closed with a 2-hole rubber stopper carrying a glass siphon for withdrawal of solution and an air inlet, the latter protected by a soda-lime tube to exclude $CO_2$. Two 4-liter reservoir bottles were filled from this stock, and were kept on an overhead shelf suspended from the frame of the greenhouse. Standard acid solution was delivered to the acid burette (B, fig. 1) of each absorption apparatus by means of glass tubing with rubber connections for flexibility. The reservoir bottles were stoppered in the same way as was the large stock bottle.

The standard alkali solution.—The standard barium hydroxide solution was prepared by dissolving "Baker's Analyzed" barium hydrate crystals in distilled water at the rate of 31.554 gm. per liter of solution with addition of 4.5 gm. "Baker's Analyzed" barium chloride per liter. The latter was added to reduce the solution and hydrolysis of the $BaCO_3$ which was present at the time titrations were made. An 18-liter stock bottle of this alkali solution was prepared at the beginning of the experiments, and this was sufficient for all of the work. The bottle was closed like the stock bottle for the standard acid solution. The small amount of barium carbonate present (there is usually some carbonate in barium hydroxide) was first allowed to settle and then the clear solution was siphoned into reservoir bottles like those used for the standard acid solution and similarly placed. From these reservoirs the solution was delivered to the charging burettes by means of a branching system of glass tubing with short rubber connections for necessary flexibility, as shown by the diagram in figure 2, described below. All air inlets into the distributing system for standard alkali solution were provided with soda-lime tubes, to prevent the entrance of atmospheric $CO_2$. That no $CO_2$ entered the system was shown by repeated tests of the solution as delivered by the burettes, titrations being made with standard acid solution and phenolphthalein.

Charging the absorption apparatus.—The charging burettes were arranged to receive and deliver standard alkali solution without its coming into contact with atmospheric $CO_2$. Their arrangement is shown in figure 2. The supply tube (T) leading from the reservoir bottle (R) is connected to the recurved tube (C) of an automatic burette (B) through the 2-way stopcock (CA). Solution flows into the burette when the cock is in the position shown and when the cock is turned half-way around solution is discharged through the tip A. Air is allowed to escape from or enter the burette through the opening above, which is fitted with a 1-hole rubber stopper bearing a soda-lime tube (G).

When an absorption tower was to be charged with alkali solution a small amount of solution was first allowed to run out of the burette into a beaker, to remove what had been in the tip (A), in contact with the air. The burette was then lowered slightly and the tip was connected immediately with the solution inlet of the absorption tower (J), after which the desired amount of solution was allowed to run into the tower. After closing the 2-way cock and disconnecting and raising the burette a small amount of $CO_2$-free water was introduced into the solution inlet before this was closed, to carry down all the solution measured from the burette. It was found that the possibility of error was reduced if the excess of standard alkali solution did not greatly exceed the equivalent of 3 or 4 cc. of the standard acid solution.

Repeated checks were made in the following manner, to test the accuracy of the method of titration within the apparatus. Some of the $BaCO_3$ from a previous experimental test was allowed to remain in the titration bottle (G, fig. 1), after the excess alkali had been neutralized and most of the liquid had been siphoned out. A measured quantity of standard alkali solution was then introduced into the absorption tower in the manner described above, and was washed down into the titration bottle with distilled water after a few drops of phenolphthalein had been added. The water for washing was introduced into the top of the tower by connecting a tube leading from a reservoir of water on the shelf above the apparatus, to the solution inlet (J, fig. 1). Ordinarily a total of 150 cc. of water, introduced in several portions, was sufficient to remove all traces of alkali from the tower, as indicated by the disappearance of pink color from the beads in the tower. The alkali solution thus introduced into the titration bottle was titrated immediately with the standard acid solution. The results of these titrations in the apparatus, in the presence of the $BaCO_3$ mentioned above, agreed with other results obtained with a similar quantity of the standard alkali solution, taken directly from the charging burette (B, figure 2) into a flask containing 150 cc. of distilled water, and titrating immediately with standard acid solution from a burette separate from the apparatus, using phenolphthalein as an indicator. This agreement of results indicates that $BaCO_3$ is not a source of error in this sort of titration, [which is in agreement with statements by TREADWELL and HALL (**44**, p. 485) and BERGMAN (**2**).] These repeated tests, both inside and outside the apparatus, served also as checks on the constancy of the standard alkali solution.

TITRATION.—As is evident from what has been said, titration was accomplished in the presence of the $BaCO_3$ that had been formed by the reaction of the absorbed $CO_2$ and the $Ba(OH)_2$ of the alkali solution in the absorp-

tion tower. At the end of a test interval, the solution in the tower was washed down into the titration bottle (G, figure 1), in the manner previously described. Very nearly the same amount of distilled water was used in each case. The water for washing was guarded against contact with atmospheric $CO_2$ by a soda-lime tube in the air inlet of the water-reservoir bottle.

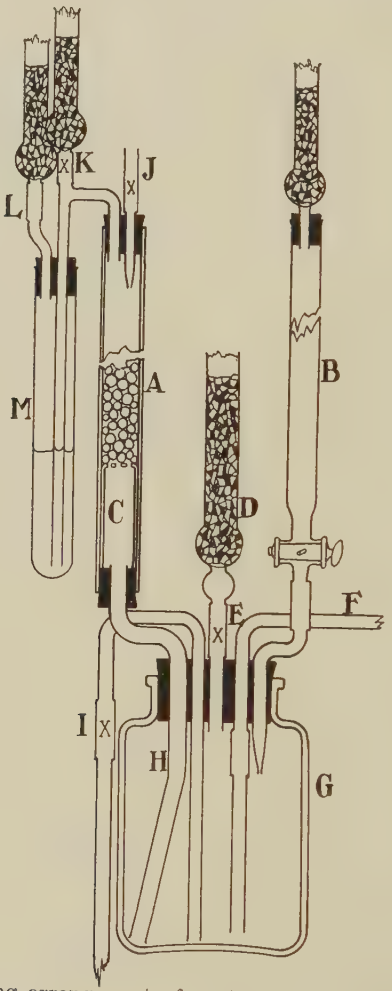


FIG. 1. Diagram showing arrangement of parts of $CO_2$-absorption apparatus. For description see text.

Standard acid solution was added drop by drop, with constant shaking of the titration bottle, to avoid any local excess of acid, which might react with the $BaCO_3$. During the process of washing down and titrating, which

required about five minutes, the gas stream continued bubbling through the solution in the titration bottle. Consequently the only loss of $CO_2$ which occurred took place while the neutralized solution was being discharged and the apparatus was being washed, preparatory to the addition of a new charge of alkali solution. This slight loss was obviously insignificant.

THE CULTURES.—Each culture consisted of 100 seedlings in 250 cc. of standard nutrient solution contained in a 300-cc. flask. The standard nutrient solution was SHIVE'S solution R5C2 (**39**), calculated to have an

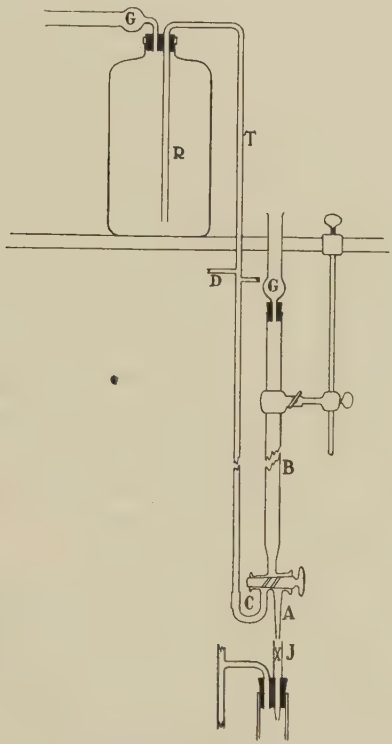


FIG. 2. Diagram showing arrangement of burette and reservoir for charging the $CO_2$-absorption apparatus. For description see text.

osmotic value of 0.175 atmosphere. Each liter contained 0.1228 gm. of $Ca(NO_3)_2$, 0.2452 gm. of $KH_2PO_4$ and 0.3698 gm. of $MgSO_4$; no iron was added. Eight liters of stock nutrient solution were prepared, 10 times as concentrated as was required. The salts used were from unopened bottles originally prepared in connection with the cooperative experiments planned by the Division of Biology and Agriculture of the National Research Council (**27**), by the Powers-Weightman-Rosengarten Co. The concentrated stock nutrient solution was prepared by dissolving the three salts together, to form

a measured volume of solution, rather than by mixing separate standardized single-salt solutions. No precipitate appeared.

When a set of five cultures was to be started the requisite volume of solution was made up by diluting 125 cc. of the stock solution to 1250 cc., 250 cc. of which was placed in each of the five culture flasks, which were numbered and stoppered and then distributed in the five temperature chambers, where each flask and its contents soon came to the temperature of the chamber. Then the 1,000 germinated seeds that had been for 42 hours in water in the germination flask in the middle chamber (19.5°) were spread out in a glass pan, from which they were transferred one by one (with bone-tipped forceps) to the culture flasks, which were removed from their chambers and remained at room temperature only for the 20 minutes required for selection and transfer. In order that the five 100-seedling lots might be as nearly alike as possible, that every lot might contain about the same proportions of somewhat larger, somewhat smaller seeds, etc. (although, as has been said, the 500 seedlings selected for a set of cultures were very nearly alike), the five lots were not selected consecutively; instead of that, 10 or 20 seedlings were transferred to one flask, then a like number to another flask and so on till every flask had received its 10 or 20 seedlings, after which this procedure was repeated in rotation, till every flask had its full quota of 100 seedlings. As soon as the transfer had been completed each flask was returned to the chamber in which it and its solution had previously been warmed or cooled, being immediately placed in connection with the gas line (with stopper set firmly in place and flask clamp tightened to insure a permanent seal) and the flow of gas mixture began.

At the close of a series of experiments (*i.e.*, immediately after the titration for the fifth test interval) the culture flasks were removed from the apparatus and their seedlings were examined with respect to the growth that had occurred. The seedlings were then discarded and the flasks were scrubbed with hot water, rinsed with distilled water and thoroughly dried, being then ready for another series of experiments. No special procedure was adopted for the sterilization of flasks and nutrient solution, nor were the wheat seeds disinfected before being placed in the germination flask. There was no evidence of growth of fungi or bacteria in any of the cultures, even at the higher temperatures.

Changes in the culture solution in the experiment period were ignored, as has been said. That such changes must have occurred, because of absorption and excretion, was indicated by a few brief studies with seedlings in nutrient solutions in which other seedlings had previously been grown for a 24-hour period, but these changes were found to be slight, probably because of the large volume of solution used for each culture and the short duration of the experiment period.

MEASUREMENTS OF $CO_2$ PRODUCTION.—Measurements of $CO_2$ production were begun 2 hours after each series of culture flasks received their seedlings, or approximately 1.5 hour after the cultures were placed under the experimental conditions. At the end of each test interval the total production of $CO_2$ for that interval (the amount of $CO_2$ that had been absorbed in the absorption apparatus during the interval) was ascertained by titration. About half an hour was required to make the five titrations, which were made always in the same order from the lowest to the highest temperature, so that the intervals were very nearly the same for the different experiments in a series. The deviation from the stated time of observation was usually less than 15 minutes; in a few cases, however, it was greater, sometimes as much as 45 minutes. The actual length of the time interval referred to by each titration record was recorded in every instance, however, and the mean hourly rate of $CO_2$ production was computed. These mean hourly rates for the five test intervals of each experiment are the numerical data on $CO_2$ production with which we shall deal when the results of these experiments are presented farther on.

OBSERVATIONS ON GROWTH.—At the end of each experiment, 48 hours after the seedlings had been placed in their experimental environment, the seedlings were removed from the culture solutions and measurements of growth were made. Although the roots elongated more rapidly than the shoots their measurement presented so many difficulties (especially because the number of roots per seedling was variable) that growth records were based on shoot elongation alone. Shoot elongation also was variable, especially at the higher temperatures, and many seedlings made so little growth in the experiment period that actual measurement was not practicable. After considerable study the following procedure was adopted for securing numerical indices of shoot elongation. All shoots that could be measured with satisfactory accuracy were measured to the nearest 0.2 millimeter, using a millimeter scale, and the average of the ten longest ones in an experiment was taken as the measure of the growth rate for that experiment. Of course these indices of growth rate are not precise, but they are sufficiently consistent to be useful, as will be seen later.

THE COMPUTATION OF AVERAGES.—The total amount of $CO_2$ collected during an interval was first expressed in terms of the equivalent volume of standard acid solution, in cubic centimeters, and this value was then multiplied by the standard factor 2.49, for 1 cc. of standard acid solution had been shown to be equivalent to 2.49 mg. of $CO_2$. The resulting product was the number of milligrams of $CO_2$ produced by the given culture in the given interval. This product was next divided by the length of the corresponding interval, expressed in hours, to give the mean hourly rate of $CO_2$ production for the interval. Five of these mean hourly rates were

thus secured for each experiment and 25 for each experiment series. In one or two instances where the number of seedlings per culture was not 100 the mean rates were all computed to represent 100 seedlings, which they did actually represent in most cases.

A preliminary, point-to-point graph was drawn to represent the five mean rates for each interval, as these differed from temperature to temperature in the same experiment series, one graph for the first interval, another for the second, etc. The ordinates of these preliminary graphs were the mean hourly rates of $CO_2$ production and the abscissas represented the corresponding temperatures, derived from thermograph or thermometer records. Each experiment series thus gave five graphs, one for each of the consecutive test intervals, and each group of graphs represented one of the 12 different oxygen-nitrogen mixtures used. Since the actual temperature means were not always exactly the standard maintained temperatures that they represented, the ordinates of each graph were corrected where necessary, to correspond more closely to the requisite temperatures, by simply measuring the actual ordinates for those temperatures (10°, 15°, 20°, 25°, and 30°) and employing the resulting values instead of the actual mean rates from which the graph had been constructed. By these procedures a mean hourly rate of $CO_2$ production by 100 seedlings was obtained for each of the five required temperatures and for each of the five test intervals in every experiment series, some slight discrepancies being thus avoided while all series were brought into conformity with regard to the five temperature values. Because every experiment series was repeated at lest once there were two or more mean hourly rates of $CO_2$ production for each of the 60 different combinations of temperature and oxygen pressure for each interval and these were finally averaged for each combination.

For the entire experiment period the total amounts of $CO_2$ produced with each combination of temperature and oxygen pressure were ascertained for each experiment, and these totals were finally averaged for each group of like experiments. Sixty average totals were thus secured, one for each combination of temperature and oxygen pressure used.

## Results and discussion

### General nature of the results

The main results of this study will now be presented, with notes on their interpretation. It is to be borne in mind that the conclusions reached are applicable only to the specific sets of internal and external conditions that prevailed in the tests. The data presented are to be studied to bring out any relations that may appear among five kinds of variables, always

with reference to the conditions of the internal and external background of the experiments, which have been described. These variables are: (1) maintained temperature, (2) partial pressure of oxygen, (3) relative stage in the progress of seedling development (indicated in each experiment by the interval number), (4) rate of $CO_2$ production and (5) rate of shoot elongation. Expressed in another way, the results are to be considered with reference to the manner in which the rates of $CO_2$ production and of shoot elongation differ, from interval to interval in the same experiment, and from experiment to experiment in relation to the several combinations of maintained temperature and maintained oxygen pressure. There are to be considered 12 different partial pressures of oxygen (the total gas pressure being always 1 atmosphere) for each of the five different maintained temperatures, and each of the 60 different combinations of temperature and oxygen pressure is to be considered with regard to each of the five consecutive test intervals of each experiment.

### No evidence of aging of seeds

The experiment series with partial pressure of oxygen about like that of ordinary air (20 per cent.) was carried out seven times, as chronologically numbered series 1, 2, 3, 4, 23, 37 and 38. (Series 5–22 and 24–36 had other oxygen pressures.) The first four series were carried out between December 10 and December 18, 1928, series 23 was carried out in February, 1929, and the last two were carried out between March 20 and March 24, 1929. The actual partial pressures of oxygen for these series were: series 1, 2 and 3, 21.3 per cent.; series 4, 20.4 per cent.; series 23, 20.0 per cent.; series 37 and 38, 18.9 per cent.; but these differences are not large enough to be significant. A study of the results of these series (brought together in table I) fails to reveal any consistent suggestion that the seedlings of series 1 or 2 may have been initially different from those of series 37 or 38 and leads to the conclusion that the stock of seed used in this study did not alter significantly during the progress of the work. It is also clear from the value of table I that the standard germination technique was not significantly altered as the work went on. These are considerations of the utmost importance in studies of this kind.

### $CO_2$ production in the several observation intervals and in the entire experiment period

The tabulated values.—The 60 average rates of $CO_2$ production for each of the five intervals are represented in table II. The five horizontal subdivisions of the table represent the five test intervals, which are numbered consecutively and designated in the following manner: 1st interval,

## TABLE I

COMPARISON OF RESULTS FROM EXPERIMENT SERIES NEAR BEGINNING AND NEAR END OF STUDY, WITH OXYGEN PRESSURE ABOUT 20 PER CENT.

| EXPERIMENT SERIES NO. | DATES OF EXPERIMENT | TEST INTERVAL | MEAN HOURLY RATE OF $CO_2$ PRODUCTION (100 SEEDLINGS) FOR TEMPERATURE OF | | | | |
|---|---|---|---|---|---|---|---|
| | | | 10° C. | 15° C. | 20° C. | 25° C. | 30° C. |
| | | | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| 1 | Dec. 12–14 1928 | 1 | 0.33 | 0.55 | 0.70 | 0.88 | 1.25 |
| | | 2 | 0.34 | 0.62 | 0.77 | 1.14 | 1.88 |
| | | 3 | 0.36 | 0.77 | 1.07 | 1.37 | 2.13 |
| | | 4 | 0.39 | 0.94 | 1.18 | 1.76 | 2.78 |
| | | 5 | 0.46 | 1.12 | 1.50 | 2.26 | 3.63 |
| 2 | Dec. 14–16 1928 | 1 | | 0.61 | 0.83 | 0.87 | 1.37 |
| | | 2 | | 0.60 | 0.85 | 1.15 | 1.93 |
| | | 3 | 0.37 | 0.76 | 1.03 | 1.35 | 2.38 |
| | | 4 | 0.47 | 0.93 | 1.29 | 1.75 | 3.27 |
| | | 5 | 0.49 | 1.08 | 1.69 | 2.34 | 3.82 |
| 3 | Dec. 16–18 1928 | 1 | 0.36 | 0.78 | 0.95 | 1.10 | 1.39 |
| | | 2 | 0.42 | 0.77 | 0.93 | 1.40 | 1.78 |
| | | 3 | 0.52 | 0.83 | 1.09 | 1.59 | 2.47 |
| | | 4 | 0.66 | 1.01 | 1.31 | 2.04 | 3.57 |
| | | 5 | 0.73 | 1.19 | 1.58 | 2.72 | 4.50 |
| 4 | Dec. 18–20 1928 | 1 | 0.40 | 0.74 | 0.84 | 0.95 | 1.49 |
| | | 2 | 0.39 | 0.72 | 0.87 | 1.08 | 1.81 |
| | | 3 | 0.51 | 0.84 | 1.06 | 1.42 | 2.37 |
| | | 4 | 0.58 | 0.98 | 1.41 | 2.07 | 3.49 |
| | | 5 | 0.68 | 1.22 | 1.69 | 2.49 | 4.03 |
| 23 | Feb. 12 1929 | 1 | 0.35 | 0.60 | 0.77 | 1.02 | 1.19 |
| | | 2 | 0.42 | 0.62 | 0.78 | 1.12 | 1.63 |
| | | 3 | 0.45 | 0.73 | 1.02 | 1.61 | 2.32 |
| 37 | Mar. 20–22 1929 | 1 | 0.44 | 0.62 | 0.82 | 0.96 | 1.23 |
| | | 2 | 0.46 | 0.62 | 0.81 | 1.07 | 1.64 |
| | | 3 | 0.48 | 0.71 | 1.04 | 1.40 | 2.14 |
| | | 4 | 0.54 | 0.91 | 1.29 | 1.77 | 3.01 |
| | | 5 | 0.63 | 1.22 | 1.71 | 2.33 | 4.00 |
| 38 | Mar. 22–24 1929 | 1 | 0.43 | 0.52 | 0.80 | 0.87 | 1.20 |
| | | 2 | 0.41 | 0.52 | 0.89 | 1.18 | 1.64 |
| | | 3 | 0.47 | 0.73 | 1.16 | 1.47 | 2.14 |
| | | 4 | 0.54 | 0.88 | 1.50 | 1.98 | 3.21 |
| | | 5 | 0.68 | 1.09 | 1.87 | 2.51 | |

Note.—Experiment series 23 was continued for three intervals only. The two blanks for series 2 and one for series 38 indicate that the data were lost.

the four hours following the close of the adjustment period (*i.e.* the 3rd to the 6th hour, inclusive, after transfer of seedlings to culture flask); 2nd interval, the next 6 hours (7th to 12th hour of the experimental period); 3rd interval, the next 12 hours (13th to 24th hour); 4th interval, the next

## TABLE II

AVERAGE RATES OF $CO_2$ PRODUCTION BY 100 SEEDLINGS FOR EACH TESTED COMBINATION OF TEMPERATURE AND OXYGEN PRESSURE IN EACH OBSERVATION INTERVAL

| INTERVALS | TEMPERATURE | AVERAGE MEAN HOURLY RATE OF $CO_2$ PRODUCTION WITH OXYGEN PRESSURE OF | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.6 PER CENT. | 3.1 PER CENT. | 6.3 PER CENT. | 9.8 PER CENT. | 16.0 PER CENT. | 20.0 PER CENT. | 30.0 PER CENT. | 50.0 PER CENT. | 75.0 PER CENT. | 90.0 PER CENT. | 95.0 PER CENT. | 98.3 PER CENT. |
| | *deg. C.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| 1ST | 10 | 0.36 | 0.38 | 0.38 | 0.42 | 0.44 | 0.39 | 0.44 | 0.45 | **0.47** | 0.46 | 0.46 | 0.46 |
| TEST | 15 | 0.46 | 0.55 | 0.61 | 0.56 | 0.58 | 0.63 | 0.71 | 0.74 | 0.82 | **0.84** | 0.75 | **0.71** |
| INTERVAL | 20 | 0.63 | 0.73 | 0.73 | 0.86 | 0.76 | 0.82 | 0.83 | 0.94 | 1.10 | **1.17** | 1.02 | 0.98 |
| (3RD TO | 25 | 0.74 | 0.88 | 0.93 | 1.02 | 0.97 | 0.95 | 1.05 | 1.20 | 1.38 | **1.50** | 1.29 | 1.23 |
| 6TH HOUR, INCLUSIVE) | 30 | 0.96 | 1.03 | 1.16 | 1.21 | 1.23 | 1.30 | 1.34 | 1.60 | 1.91 | **2.08** | 1.87 | 1.55 |
| 2ND | 10 | 0.26 | 0.33 | 0.31 | 0.34 | 0.41 | 0.41 | 0.54 | 0.56 | 0.59 | **0.64** | 0.52 | 0.53 |
| TEST | 15 | 0.41 | 0.52 | 0.56 | 0.54 | 0.56 | 0.64 | 0.72 | 0.85 | 0.98 | **1.04** | 0.93 | 0.92 |
| INTERVAL | 20 | 0.61 | 0.73 | 0.77 | 0.80 | 0.83 | 0.84 | 0.94 | 1.05 | 1.37 | **1.57** | 1.38 | 1.36 |
| (7TH TO | 25 | 0.79 | 1.00 | 1.14 | 1.37 | 1.20 | 1.16 | 1.24 | 1.44 | 1.81 | **2.04** | 1.74 | 1.80 |
| 12TH HOUR, INCLUSIVE) | 30 | 1.11 | 1.32 | 1.72 | 1.86 | 1.75 | 1.76 | 1.69 | 2.02 | 2.56 | **2.88** | 2.55 | 2.42 |
| 3RD | 10 | 0.27 | 0.30 | 0.34 | 0.33 | 0.44 | 0.45 | 0.59 | 0.69 | **0.75** | 0.73 | 0.66 | 0.63 |
| TEST | 15 | 0.46 | 0.62 | 0.61 | 0.52 | 0.62 | 0.78 | 0.83 | 0.97 | 1.30 | 1.42 | **1.55** | 1.25 |
| INTERVAL | 20 | 0.66 | 1.00 | 1.11 | 1.06 | 1.08 | 1.07 | 1.21 | 1.43 | 1.98 | **2.41** | 2.20 | 2.24 |
| (13TH TO | 25 | 0.93 | 1.52 | 1.81 | 1.95 | 1.74 | 1.46 | 1.67 | 1.93 | 2.80 | **3.32** | 3.16 | 3.18 |
| 24TH HOUR, INCLUSIVE) | 30 | 1.38 | 1.97 | 2.29 | 2.36 | 2.62 | 2.28 | 2.25 | 2.88 | 3.88 | **4.65** | 4.54 | 4.07 |
| 4TH | 10 | 0.28 | 0.35 | 0.37 | 0.36 | 0.51 | 0.53 | 0.70 | 0.86 | 0.82 | **0.89** | 0.76 | 0.77 |
| TEST | 15 | 0.47 | 0.88 | 0.82 | 0.70 | 0.75 | 0.94 | 1.04 | 1.25 | 1.66 | 1.91 | **2.04** | 1.52 |
| INTERVAL | 20 | 0.70 | 1.30 | 1.37 | 1.18 | 1.17 | 1.33 | 1.46 | 1.88 | 2.82 | 3.49 | **3.60** | 3.54 |
| (25TH TO | 25 | 1.03 | 1.85 | 2.06 | 2.31 | 2.21 | 1.90 | 2.00 | 2.75 | 3.99 | 4.56 | **5.14** | 4.65 |
| 36TH HOUR, INCLUSIVE) | 30 | 1.41 | 2.38 | 2.56 | 3.19 | 3.76 | 3.24 | 3.11 | 4.63 | 5.22 | 5.93 | **6.18** | 5.47 |
| 5TH | 10 | 0.29 | 0.44 | 0.45 | 0.37 | 0.60 | 0.61 | 0.83 | 1.05 | 1.06 | **1.15** | 1.11 | 1.04 |
| TEST | 15 | 0.50 | 1.01 | 1.02 | 0.87 | 0.91 | 1.15 | 1.34 | 1.71 | 2.41 | 2.90 | **3.34** | 2.55 |
| INTERVAL | 20 | 0.80 | 1.41 | 1.54 | 1.55 | 1.43 | 1.67 | 1.83 | 2.58 | 3.81 | 4.70 | **5.01** | 4.86 |
| (37TH TO | 25 | 1.23 | 2.14 | 2.30 | 2.93 | 2.97 | 2.45 | 2.75 | 3.87 | 4.94 | 5.66 | **6.30** | 5.72 |
| 48TH HOUR INCLUSIVE) | 30 | 1.48 | 2.70 | 3.08 | 3.72 | 4.66 | 4.00 | 4.34 | 5.81 | 6.14 | 6.67 | **6.87** | 6.38 |
| NUMBER OF EXPERIMENTS | | 3 | 2 | 2 | 2 | 4 | 7 | 3 | 2 | 4 | 3 | 3 | 3 |

12 hours (24th to 36th hour); 5th interval, the last 12 hours of the experiment period (37th to 48th hour). Each line of the table shows values for a single temperature and each column shows values for a single oxygen pressure. The number of experiments on which the values of each column are based is shown at the bottom of the column. The probable errors of the averages were computed in a large number of cases by BESSEL'S (30) formula, and they were found in most cases to be less than 4 per cent. of the average itself; in a very few cases the probable error was about 6 per cent. of the average. It is probably safe to suppose that the lowest values given in table II represent the facts within plus or minus 0.02 or 0.03 mg. and that the highest values are thus representative within plus or minus 0.4 or 0.5 mg.

From the averages given in table II, the most obvious facts to be noted are that these experiments gave rates of $CO_2$ production varying in magnitude from below 0.3 mg. to nearly 7.0 mg. per hour, for 100 seedlings, and that the magnitudes of the rates are clearly related to the temperature-oxygen combination used and to the developmental phase of the plantlets.

PROGRESSIVE INCREASE IN $CO_2$ PRODUCTION THROUGHOUT THE EXPERIMENT PERIOD.—It is generally evident from table II that the rate of $CO_2$ production for any combination of temperature and oxygen pressure became progressively greater with time; the rate for a later interval generally more or less exceeds the corresponding rate for any earlier interval in the same experiment. This would be expected, for the rate here considered should generally be greater as development of the plantlets proceeds through its earlier phases. The developmental progress of $CO_2$ production must have been very slow at the inception of germination and this progress in the experiment period fails to be indicated or was only slightly marked for combinations of low temperature and low oxygen pressure. This progress is generally well shown, however, for combinations of low temperatures and high oxygen pressure and for combinations of high temperatures and any oxygen pressure tested, being most pronounced for the combination of 30° and 90 or 95 per cent. of oxygen in the gas mixture.

The time graphs of figures 3 and 4 are representative samples of a complete series of graphs representing all the data in table II. Figure 3 shows the five time graphs, one for each of the temperatures tested, for each of six representative oxygen pressures; namely, 9.8, 20, 50, 75, 90 and 98.3 per cent. Each section of the figure shows the numerical data for $CO_2$ production (ordinates) for each of the observation intervals (abscissae), there being five graphs in each case, one for each of the maintained temperatures used. The temperature represented is shown at the right-hand end of each graph. Each of these single graphs thus shows the time march of $CO_2$ production from the first interval (3rd to 6th hour after the

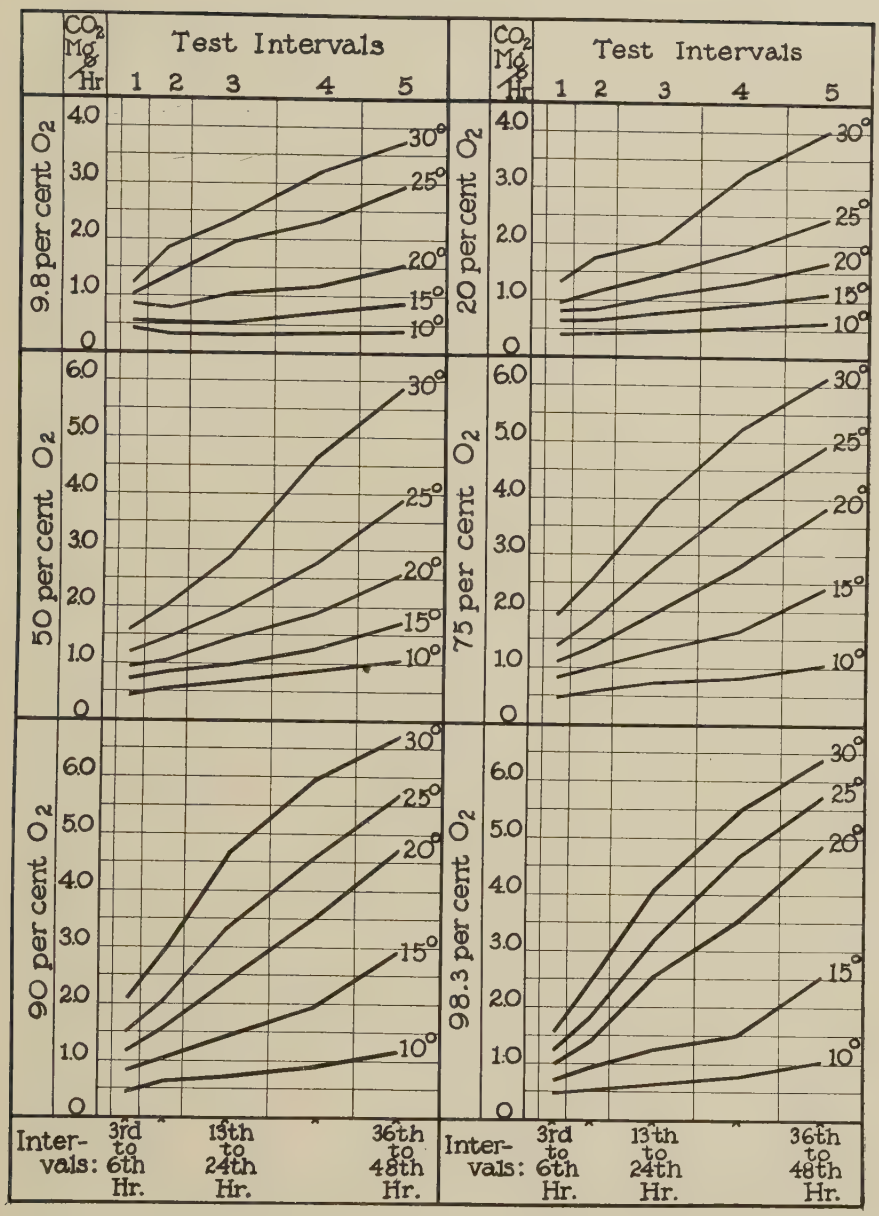


FIG. 3. Representative time graphs showing the march of $CO_2$ production during the experiment period for each temperature as related to oxygen pressure. These are arranged in groups, each group representing a different oxygen percentage.

start of the experiment) to the fifth interval (37th to 48th hour). In each of the time graphs (including those of figure 4 as well as those of figure 3) the abscissa for any point represents the middle of the observation interval in question; for example, data for the second interval (7th to 12th hour of the period) are plotted as referring to the end of the 9th hour.

In no section of the series of graphs represented by figure 3 is there any crossing or coincidence of the graphs; with every oxygen pressure tested the five-degree temperature intervals were amply great to insure perfectly distinct graphs of this sort and it is apparent that much narrower temperature intervals might have been employed without the introduction of confusion due to unexplained variability among the lots of seedlings. Of course the five graphs for any oxygen pressure approach one another for the shorter abscissae, since the rates of $CO_2$ production must all have approached zero at the beginning of the germination period, no matter what combination of temperature and oxygen pressure was used.

The graphs of figure 4 are in part the same graphs as are shown in figure 3. In figure 3 there is for each one of six representative *oxygen pressures* a group of five time graphs (*for the five temperatures*) and each section of the figure represents *a single oxygen pressure.* In figure 4, on the other hand, there is for each of the five *temperatures* a group of four or five time graphs (*for representative oxygen presures*) and each section of this figure represents *a single temperature.* By the first arrangement the graphs are grouped according to oxygen presure while they are grouped according to temperature by the second arrangement. In figure 4 both the lowest and the highest graph are shown for each temperature, with one or two intervening graphs for representative oxygen pressures between the pressure for the lowest and that for the highest graph. These are all full lines. For 10° they are for oxygen pressures of 0.6, 20 and 90 per cent. and for the other temperatures they are for 0.6, 20, 50 and 95 per cent. (In the few instances where the same graph is not highest with respect to all intervals the graph shown as highest is the one that is highest for the fifth interval.) In addition, the graph for the oxygen pressure of 98.3 per cent. is shown in every case, its line being broken to distinguish it more readily from the other graphs.

Some temperature-oxygen combinations failed to show a regular increase in the rate of $CO_2$ production from interval to interval and these exceptions are of special interest. They are listed here for the sake of inspection. Each combination is shown, in the following lists, by its temperature and its oxygen-percentage value separated by a comma, and the value in parenthesis is the difference (in hundredths of a milligram) between the two average rates considered. For the following thirteen combinations of temperature and oxygen pressure the average for the second interval

does not exceed that for the first: 10°, 0.6 (10); 10°, 3.1 (5); 10°, 6.3 (7); 10°, 9.8 (8); 10°, 16 (3); 15°, 0.6 (5); 15°, 3.1 (3); 15°, 6.3 (5); 15°, 9.8 (2); 15°, 16 (2); 20°, 0.6 (2); 20°, 3.1 (0); 20°, 9.8 (6). For the following eight combinations the average for the third interval does not exceed that for the first: 10°, 0.6 (9); 10°, 3.1 (8); 10°, 6.3 (4); 10°, 9.8 (9);

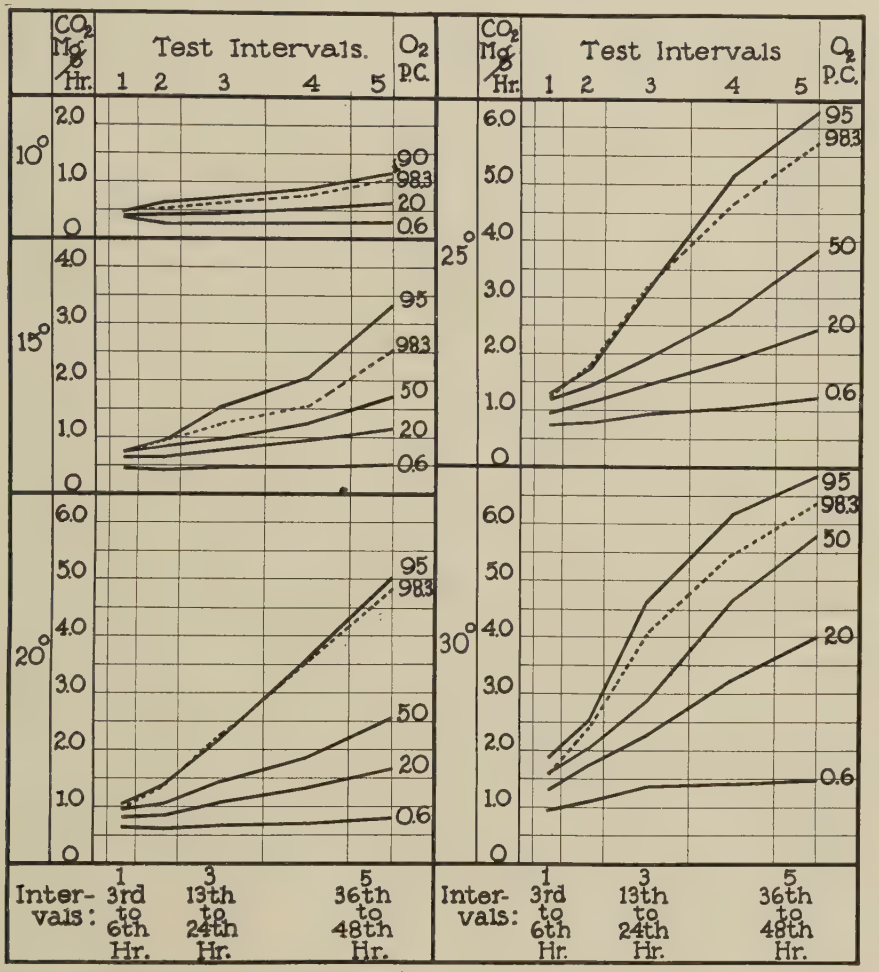


FIG. 4. Representative time graphs showing the march of $CO_2$ production during the experiment period for each oxygen pressure as related to temperature. These are arranged in groups corresponding to the several temperatures, each group representing a different temperature.

10°, 16 (0); 15°, 0.6 (0); 15°, 6.3 (0); 15°, 9.8 (4). For the following four combinations the rate for the fourth interval does not exceed that for the first: 10°, 0.6 (8); 10°, 3.1 (3); 10°, 6.3 (1); 10°, 9.8 (6). Only for

the combination of 10° and 0.6 per cent. does the rate for the fifth interval fail to exceed that for the first. These exceptions are all confined to temperatures of 20° or lower and to oxygen pressures of 16 per cent. or lower and they correspond to environmental complexes that gave relatively low rates of $CO_2$ production in any interval. In some of these instances the exceptions may be due to possible errors in observation, which were of course relatively larger for these lowest rates, but many of them appear to be significant, suggesting that the effect of the environmental change from germination flask to culture flask (or the lagging influence of the environmental conditions of the germination period extending into the experimental period) required more or less of the experiment period in which to disappear. It is possible that the effect of the environmental change itself may have been important in determining the higher rate of $CO_2$ production in the earlier test intervals, in the cases where this occurred. It has been mentioned in various connections in the literature that a sudden change in the environment may result in a stimulation or retardation of activity, either of which may be opposite in tendency from the direct effect of the conditions prevailing after the change. A recent notable example of this is contained in a paper by Parija (**34**), in which the graphs illustrate pronounced temporary increases in $CO_2$ production by apples when they were subjected to a change from one atmospheric environment to another with a lower oxygen percentage, and a temporary reduction in $CO_2$ production for an environmental change of the opposite description, for changes between several different oxygen percentages, including ordinary air, pure oxygen, pure nitrogen, and percentages of oxygen from 3 to 9 at intervals of 2 per cent.

In this connection we may neglect the data for the first interval and consider the period of transfer and adjustment as of 8 rather than 2 hours. When that is done an examination of table II shows only three exceptions of the sort here considered; only for the three combinations, 10°, 3.1 (3); 10°, 9.8 (1) and 15°, 9.8 (2), were the rates for the second interval greater than those for the third and these exceptions are negligible. Rates for the second interval were in all cases smaller than the corresponding rates for the fourth or fifth.

Because of the progressive increase in the rate of $CO_2$ production throughout the period, the time graphs generally slope upward from low ordinates at the left to higher ones at the right. The mean rate of upward slope is usually greater with higher temperature than with lower, for any oxygen pressure. It is usually greater with higher oxygen pressure than with lower, for any temperature, but generally there is indicated an optimal oxygen pressure below 98.3 per cent. In no case does a graph bend downward at the right, which indicates that no maximum rate of $CO_2$ production

was attained in the experiment period with any of these complexes of conditions. In some cases the graphs beyond the second interval are nearly rectilinear (*e.g.*, that for 25° and 20 per cent.) in other cases they are somewhat concave upward in the region of the later intervals, and in still other cases they are somewhat convex upward in that region. Of course upward convexity means a decreasing acceleration of the rate of $CO_2$ production and suggests that a maximum in that rate may have been approached. This last suggestion applies especially to the graphs for 30°, a temperature that must be regarded as surely supra-optimal for health in such seedlings as these, for it is likely that pathological conditions supervened before the close of the experiment period in all cultures maintained at 30°.

In each section of figure 4 the upward slope of the time graphs is seen to be generally steeper for any temperature as the oxygen pressure is greater, until the highest graph for the given temperature is reached, but the graph for a pressure of 98.3 per cent. (broken line) regularly lies below the highest graph. The highest oxygen pressure (nearly pure oxygen) gave generally lower average rates of $CO_2$ production for any temperature and observation interval than did the slightly lower pressure that gave the corresponding highest rate for that temperature and interval. In the whole series of time graphs represented by figure 4 the only exceptions to this rule are for 10° in the first interval. The rates for 10° in the first interval are low and alike for 90, 95 and 98.3 per cent. For 25° in the second and third intervals the graph for 98.3 per cent. lies slightly above that for 95 per cent., but in these two instances the highest rates were given by an oxygen pressure of 90 per cent. and reference to table II shows that these two ordinates of the graph for 98.3 are both much smaller than the corresponding values for 90 per cent.

Special study of the combination of 20° and the oxygen pressure of 20 per cent. in the several intervals.—Special attention may be called to the behavior of the seedlings subjected to the maintained combination of 20° and 20 per cent. The values for this combination of a commonly experienced temperature and ordinary air may be considered as fairly representative of many germination tests and of what occurs when such seed as was used in this study is allowed to germinate in field plantings. These seedlings had been transferred from water to the standard nutrient solution at the beginning of the experiment but their temperature and oxygen pressure had not been significantly different during the experiment period from what these had been during the germination period. For the youngest development phase studied (1st interval, when shoots were only about 0.5 mm. long and roots were barely showing through the coleorhiza) the average mean rate of $CO_2$ production was 0.82 mg. per hour per 100

seedlings. The rate increased as time went on (being 0.84 mg. for the 2nd interval, 1.07 mg. for the 3rd interval, 1.33 mg. for the 4th interval) until it had a value of 1.67 mg. for the last interval, when the seedlings had shoots about 5 mm. long and roots about 20 mm. long. It is remarkable that this series of values, from 0.84 for the 2nd interval (say 9 hours after the beginning of the experiment), to 1.67 for the 5th interval (say 42 hours after the beginning of the experiment), shows an increase that is almost proportional to time, at the rate of about 0.025 mg. per hour.

Rates in the second interval.—Because some of the rates of $CO_2$ production in the first interval appear to show influence of the environmental change that occurred when the cultures were started, the earliest interval which is practically without such inconsistencies is the second, the data for which may be taken to represent these seedlings at a very early developmental stage. These data are shown in the second horizontal section of table II and by the graphs of figures 5 and 6.

The chart of figure 5 was planned to show how these very young seedlings (all of the same age with respect to time) exhibited different rates of $CO_2$

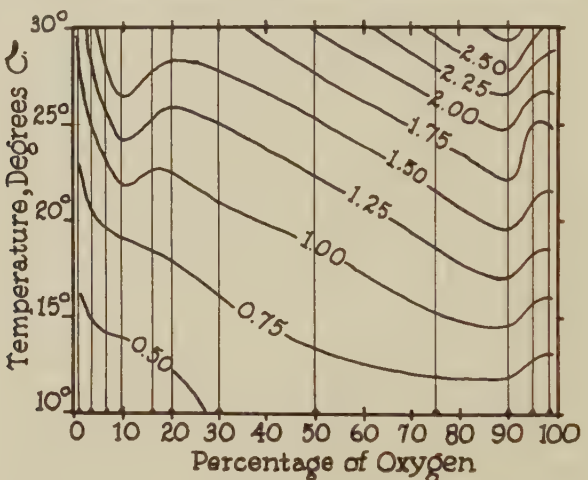


Fig. 5. Diagram showing by contours or isopleths, the relations between rate of $CO_2$ production (by 100 seedlings) and maintained temperature-oxygen combinations for the second test interval. Points of intersection of the vertical and horizontal lines represent the values given in table II. For all points on any contour the average rate of $CO_2$ production is the same, its value being indicated by the number on the contour (0.50 to 2.50).

production according to the particular maintained combinations of temperature and oxygen pressure to which they had been subjected since the cultures were started. This chart represents a solid diagram in which "east-west" distances represent progressively larger partial pressures of

oxygen, "south-north" distances represent progressively higher maintained temperatures, and altitudes (as shown by the values on the isopleths or contours) represent hourly rates of $CO_2$ production. Isopleths are shown for each rate from 0.50 mg. to 2.75 mg., by intervals of 0.25 mg. A glance at this diagram will show what average hourly rate corresponds to any tested combination of temperature and oxygen pressure, or what combinations of these factors correspond to any particular average rate, for the second observation interval. It is notable that the same rate may correspond to many different combinations of temperature and oxygen pressure. For example, the rate of 1.0 mg. per hour per 100 seedlings in the second test interval coresponds to combinations of about: 14.5° and 86 per cent., 17.5° and 58 per cent., 20° and 38 per cent., 22.75° and 18 per cent., 22° and 10 per cent., 28.5° and 0.3 per cent., etc., etc. This manner of presenting the numerical data emphasizes the generalization that neither temperature alone nor oxygen pressure alone is to be considered as the determining condition for any rate of $CO_2$ production; both of these factors must be considered and there is a broad range of combinations of them that correspond to the same rate.

It is to be noted that the isopleths of figure 5 are in general closer together (*i.e.*, the slope of the surface represented by the diagram is steeper) in the region of combinations of the higher values of the two experimental variables than in the region of combinations of the lower values. In other words, these seedlings were less sensitive, as far as the rate of $CO_2$ production is concerned, to differences between combinations of high temperature and high oxygen pressure than to differences between combinations of low temperature and low oxygen pressure.

It is interesting to observe also that, for oxygen pressures between about 20 per cent. and about 90 per cent., the temperature corresponding to any average hourly rate of $CO_2$ production is generally lower as the oxygen pressure is higher, the oxygen pressure corresponding to any rate is lower as the temperature is higher, and the relation between temperature and oxygen pressure for any average rate above about 1 mg. per hour is nearly a linear one and about the same for all these rates. Within the region of the diagram here considered (between oxygen pressure of about 20 per cent. and about 90 per cent.) the percentage value of the oxygen pressure giving any average rate of $CO_2$ production above about 1 mg. per hour is decreased by about 15 for each temperature increase of about 2°. Rates of $CO_2$ production below about 1 mg. per hour show contours with progressively lower slopes in the region of approximate linear relation between temperature and oxygen pressure, the left margin of this region being shifted to the right. The characteristic forms of the contours for the region of oxygen pressures below about 20 per cent. and for the region

of pressures above about 90 per cent. bring out other modifications which will be referred to below. Within the range of oxygen pressure between about 20 and about 90 per cent. the rate of $CO_2$ production for any pressure is shown to increase rather regularly with increase in temperature, somewhat more rapidly in the higher than in the lower temperature ranges. Also, the rate of $CO_2$ production for any temperature is shown to increase rather regularly with increase in oxygen pressure within the specified range, somewhat more rapidly for higher than for lower temperatures.

Other diagrams similar to that of figure 5, for the data of the 3rd, 4th and 5th intervals and for the experiment period as a whole are just as interesting and instructive as the one here shown. They may be readily constructed from the data given in table II.

Figure 6 presents plane graphs of the data on $CO_2$ production in the second interval. Ordinates represent average hourly rates of $CO_2$ production and abscissae represent percentages of oxygen in the gas mixture.

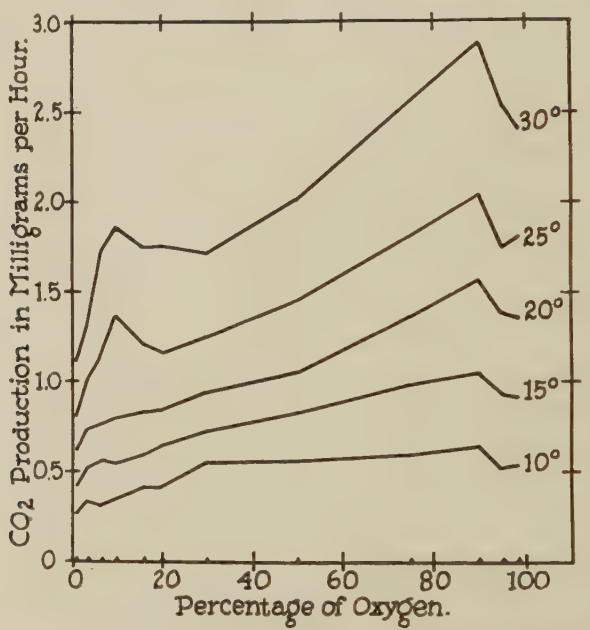


FIG. 6. Graphs of $CO_2$ production in the second observation interval, as related to oxygen pressure and to temperature. Each graph represents a different temperature.

There are five graphs in each group, one for each temperature tested.

Two graph maxima are clearly shown for 25° and 30° and suggested for 10°, 15° and 20°. For 25° and 30° the first graph maximum corresponds to a pressure of 9.8 and in all cases the second graph maximum

corresponds to a pressure of 90 per cent. Between these two graph maxima there is indicated a minimum region, the minimum itself corresponding to 20 per cent. for 25° and to 16–30 per cent. for 30°.

The existence of two graph maxima implies two optimal oxygen pressures for the given temperature, the first (lower) optimal pressure being 9.8 per cent. for 25 and 30° and the second (higher) optimal pressure being 90 per cent. The second or main optimum of pressure is clearly shown for all temperatures tested in the second interval, but the first optimum is only suggested for the three lower temperatures, for which it appears to be progressively lower with lower temperature. The double optimum of oxygen pressure is also indicated by the contours of figure 5; the graphs of figure 6 are of course simply "east-west" profiles of the area represented by figure 5. More attention will be given to this double optimum in connection with the discussion of the rates for the entire period, in the next following section.

Another aspect of the complex set of relations shown by figures 5 and 6, for the second observation interval, may be illustrated by noting that although the rate of $CO_2$ production is shown to be generally higher with higher temperature and also with higher oxygen percentage, within the ranges studied, yet the two influences are not generally equally effective. The temperature influence is most effective with an oxygen pressure of about 90 per cent. and least effective with a pressure of 0.6 per cent., but it is more effective with 10 than with 20 per cent. and less effective with 98.3 than with 90 per cent. The plantlets were able to produce $CO_2$ most rapidly, in this second interval, when the temperature effectiveness was high; that is, when a given increase in temperature gave a large increase in $CO_2$ production.

The rate of $CO_2$ production for any temperature in the second interval is shown by the plane graphs to have been nearly proportional to the oxygen pressure for a range of pressure between about 20 or 30 per cent. and about 90 per cent., the upward slope of the nearly rectilinear graphs for that range being progressively steeper with higher temperature. A similar observation has been noted from the isopleths of figure 5.

$CO_2$ PRODUCTION IN THE ENTIRE EXPERIMENT PERIOD.—The data for total $CO_2$ production in the whole experiment period are given in table III and are represented by the graphs of the first part of figure 7. The arrangement of these graphs is like that of the graphs in figure 6, ordinates being the average rates of $CO_2$ production while abscissae are oxygen pressures, with a separate graph for each of the five temperatures. The units shown at the left of the figure are for average hourly rates for the whole period; *i.e.*, each total value given in table III has been divided by 46 (the number of hours in the experiment period) to give the corresponding ordinate shown in the graphs.

## TABLE III

TOTAL $CO_2$ PRODUCTION BY 100 SEEDLINGS FOR EACH COMBINATION OF TEMPERATURE AND OXYGEN PRESSURE IN THE ENTIRE EXPERIMENT PERIOD

| TEMPERATURE | AMOUNT OF $CO_2$ PRODUCED IN 46 HOURS (BY 100 SEEDLINGS) WITH OXYGEN PRESSURE OF | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.6 PER CENT. | 3.1 PER CENT. | 6.3 PER CENT. | 9.8 PER CENT. | 16.0 PER CENT. | 20.0 PER CENT. | 30.0 PER CENT. | 50.0 PER CENT. | 75.0 PER CENT. | 90.0 PER CENT. | 95.0 PER CENT. | 98.3 PER CENT. |
| *Deg. C.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| 10 | 12.8 | 16.6 | 17.0 | 16.6 | 22.5 | 23.0 | 30.3 | 36.3 | 36.8 | **39.1** | 35.4 | 34.5 |
| 15 | 21.6 | 37.2 | 35.4 | 30.3 | 33.1 | 40.8 | 45.5 | 55.2 | 73.6 | 82.3 | **91.5** | 72.2 |
| 20 | 31.3 | 51.9 | 55.7 | 52.4 | 51.9 | 57.1 | 63.0 | 80.4 | 115.8 | 141.2 | **142.1** | 139.8 |
| 25 | 46.0 | 75.4 | 84.6 | 98.4 | 93.8 | 80.4 | 88.3 | 115.8 | 157.3 | 180.2 | **190.8** | 178.3 |
| 30 | 61.6 | 96.6 | 110.3 | 127.3 | 144.3 | 130.2 | 131.4 | 178.3 | 205.1 | **232.6** | 228.6 | 211.4 |

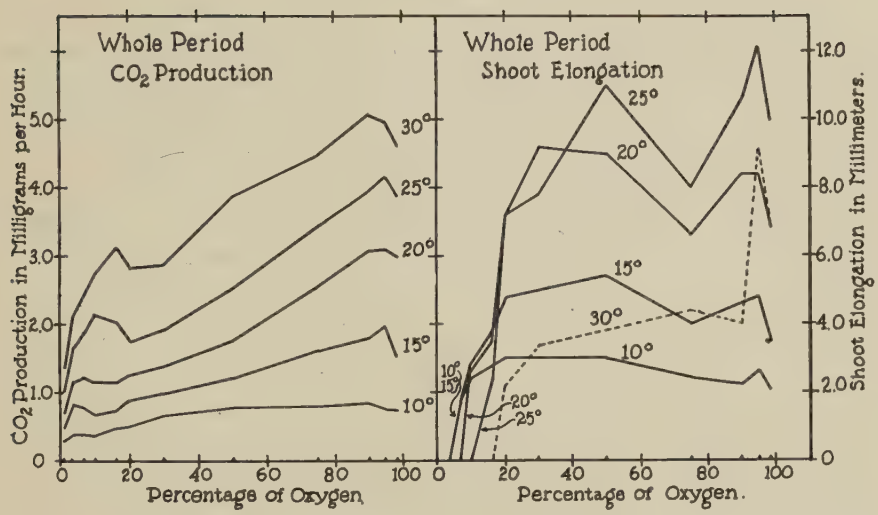


FIG. 7. Graphs of $CO_2$ production and of shoot elongation in the entire experiment period, as related to oxygen pressure and to temperature. Each graph represents a different temperature.

The graphs for the entire period are very similar to those for the second interval. The two graph maxima shown for the second interval are clearly shown here for 15°, 20°, 25° and 30° and are suggested for 10°. The portion of each graph between the subordinate minimum and the second maximum is nearly rectilinear, with the slope progressively steeper as the temperature is higher, as is true of the graphs for the higher temperatures in the second interval. The first graph maximum corresponds to higher oxygen pressure with higher temperatures; for 10° and 15° it is indicated as corresponding to 3.1 per cent., for 20° it corresponds to 9.8 per cent., and for 30° it corresponds to 16 per cent. These pressure values are those of the first or subordinate optimal pressure. The second graph maximum corresponds to a pressure of 90 or 95 per cent. for all temperatures and this slight variability appears not to be related to temperature. The abscissa of the second graph maximum is of course the main or second optimal oxygen pressure in each instance.

It is specially remarkable that the main optimal oxygen pressure is shown as below 98.3 per cent. for every temperature tested in every interval and in the whole period; this highest pressure tested was surely supra-optimal in every instance. Reference to table II shows that the main optimal oxygen pressure (the average rates for which are there shown in bold-face type) was either 90 or 95 per cent. in every case excepting 10° in the first and third intervals, for which it is shown as 75 per cent.

The subordinate graph minimum for the graphs of the whole period generally corresponds, like the first maximum, to an oxygen pressure that is somewhat higher as the temperature is higher. For 10° and 15° its abscissa is 9.8 per cent., for 20° the corresponding oxygen pressure is shown as between 9.8 and 16 per cent., for 25° its abscissa is 20 per cent. and for 30° between 20 and 30 per cent.

The existence of two maxima with an intervening minimum in graphs of the sort shown in figure 6 and the first part of figure 7 seems not to have been pointed out in the literature before and the now obscure temperature-oxygen relations that must be responsible for these peculiar features are worthy of special study. An explanation is doubtless to be sought in the physiological chemistry of respiration in such seedlings as these, but no complete and plausible hypothesis seems as yet to be possible in this connection. An apparently pertinent suggestion will be presented after the graphs for shoot elongation have been considered. As has been mentioned, these two graph maxima imply a double optimal oxygen pressure for each temperature, the first (or lower) optimal pressure being in each instance the abscissa for the first graph maximum, while the second (or higher, main) optimal pressure is the abscissa for the second graph maximum. The physiological meaning of this apparently important double optimal pressure may be made clear by a concrete example, as follows: For the whole experiment period and the temperature of 25° the oxygen pressure of 9.8 per cent. gave a much higher rate of $CO_2$ production than was given either by 6.3 per cent. or by 20 per cent., while the rates for 6.3 and 20 per cent. are shown as about alike. The rate of $CO_2$ production for 25° and 6.3 per cent. of oxygen was 84.6 mg. and that for 25° and 20 per cent. of oxygen was 80.4 mg., while that for 25° and 9.8 per cent. of oxygen was 98.4 mg.

Graphs are shown here for only the second interval and the entire period, but the data of tables II and III show that the double graph maximum and the intervening minimum occur rather regularly for other intervals than the second, though the data are not wholly consistent in this respect. In table IV are assembled the approximate oxygen-pressure values corresponding to the first maximum, the second minimum and the main maximum in each graph, including the graphs not here reproduced as well as those of figure 6 and the first part of figure 7. The data are from tables II and III.

With two exceptions (30°, first interval, and 20°, second interval) the double graph maximum, which implies a double optimum in oxygen pressure, is at least suggested for all temperatures tested in all observation intervals and in the whole period and it is clearly shown for the higher temperatures in every case. Both the first optimal pressure (correspond-

## TABLE IV

APPROXIMATE OXYGEN PRESSURES (PERCENTAGE OF OXYGEN IN THE GAS STREAM) CORRESPONDING TO FIRST OR SUBORDINATE MAXIMUM, SECOND OR SUBORDINATE MINIMUM AND SECOND OR MAIN MAXIMUM ON EACH GRAPH OF $CO_2$ PRODUCTION FOR EACH TEMPERATURE TESTED IN EACH OBSERVATION INTERVAL AND IN THE ENTIRE EXPERIMENT PERIOD. THE GRAPHS FOR THE SECOND INTERVAL AND THE ENTIRE PERIOD ARE REPRODUCED IN FIGURES 6 AND 7

| | | 10° | 15° | 20° | 25° | 30° |
|---|---|---|---|---|---|---|
| | | *per cent.* | *per cent.* | *per cent.* | *per cent.* | *per cent.* |
| 1st interval | 1st max. | 16 | 6.3 | 9.8 | 9.8 | ..... |
| | 2nd min. | 20 | 9.8 | 16 | 20 | ..... |
| | 2nd max. | 75 | 90 | 90 | 90 | 90 |
| 2nd interval | 1st max. | 3.1? | 6.3? | ..... | 9.8 | 9.8 |
| | 2nd min. | 6.3? | 9.8? | ..... | 20 | 30 |
| | 2nd max. | 90 | 90 | 90 | 90 | 90 |
| 3rd interval | 1st max. | 6.3? | 6.3 | 6.3 | 9.8 | 16 |
| | 2nd min. | 9.8? | 9.8 | 16 | 20 | 30 |
| | 2nd max. | 75 | 95 | 90 | 90 | 90 |
| 4th interval | 1st max. | 6.3? | 6.3 | 6.3 | 9.8 | 16 |
| | 2nd min. | 9.8? | 9.8 | 16 | 20 | 30 |
| | 2nd max. | 90 | 95 | 95 | 95 | 95 |
| 5th interval | 1st max. | 6.3 | 6.3 | 9.8 | 16 | 16 |
| | 2nd min. | 9.8 | 9.8 | 16 | 20 | 20 |
| | 2nd max. | 90 | 95 | 95 | 95 | 95 |
| Whole period | 1st max. | 3.1? | 3.1 | 6.3 | 9.8 | 16 |
| | 2nd min. | 9.8? | 9.8 | 9.8—16 | 20 | 20—30 |
| | 2nd max. | 90 | 95 | 90—95 | 95 | 90 |

ing to the first graph maximum) and the pressure corresponding to the second graph minimum are generally higher with higher temperature and with later intervals. Of course the pressure corresponding to the second minimum on a graph is always somewhat higher than that corresponding to the first maximum, the latter pressure being the one here called the first optimal pressure. Although some inconsistencies are to be noted, it seems clear from table IV that the double optimum of oxygen pressure for $CO_2$ production is significant and that the indications in this respect that are shown by the graphs of figures 6 and 7 are truly representative of the entire series of graphs.

The five graphs of $CO_2$ production in the whole period are also like those for the same process in the second interval in that each one has a

nearly rectilinear region lying between the second or subordinate minimum and the second or main maximum; for oxygen pressures between about 20 or 30 per cent. and about 90 per cent., $CO_2$ production was nearly proportional to oxygen pressure and the constant of proportionality (the upward slope of the graphs) is progressively greater with higher temperature, its value being apparently determined by a temperature and the current development phase of the plantlets.

### Shoot elongation in the entire experiment period

Observations on the rates of enlargement exhibited by the seedlings of the various cultures were not sufficiently precise for use in studying the several observation intervals of the experiment period but the average final length of the longest ten shoots of each culture furnishes a numerical value that may represent the corresponding total shoot elongation for the entire period. These average final lengths are given in table V. Where no value is shown, elongation was too slight to be measured by the rather crude method employed. All values in any line of the table are for the same oxygen pressure and the highest value for each pressure is designated by the use of bold-face type. An asterisk is shown at the left of the highest growth value for each temperature (highest value in each column) and a vertical line at the right brackets several values when these appear to indicate an optimal range of oxygen pressure for the temperature in question.

Besides the graphs of $CO_2$ production as related to temperature and oxygen pressure in the entire experiment period, figure 7 presents also the corresponding graphs for shoot elongation, the data for the latter being taken from table V. Ordinates of these growth graphs are indices of total shoot elongation for the entire experiment period and abscissae represent percentages of oxygen in the gas mixture. There is a graph for each temperature tested.

The graphs of shoot elongation differ from the corresponding ones of $CO_2$ production in several ways. While no main minimal oxygen pressure is shown for $CO_2$ production with any temperature, all five of the graphs of shoot elongation are seen to meet the axis of abscissae at the left. It is important to note that the highest oxygen pressure giving no measurable growth (a pressure slightly below the minimal pressure for shoot elongation) is progressively greater with higher temperatures. For 10° and 15° this is 3.1 per cent., for 20° it is 6.3 per cent., for 25° it is 9.8 per cent. and for 30° it is 16 per cent.

Each of these graphs of shoot elongation shows three low or minimal regions and two high or maximal regions on the oxygen scale, as in the case of the corresponding graphs of $CO_2$ production, but details are very

## TABLE V

AVERAGE LENGTHS OF TEN LONGEST SHOOTS IN EACH CULTURE, AT END OF EXPERIMENT, AS RELATED TO TEMPERATURE AND PARTIAL PRESSURE OF OXYGEN

| PARTIAL PRESSURE OF OXYGEN | AVERAGE FINAL LENGTH OF TEN LONGEST SHOOTS IN EACH CULTURE, FOR TEMPERATURE OF | | | | |
|---|---|---|---|---|---|
| | 10° C. | 15° C. | 20° C. | 25° C. | 30° C. |
| *per cent.* | *mm.* | *mm.* | *mm.* | *mm.* | *mm.* |
| 0.6 | ..... | ..... | ..... | ..... | ..... |
| 3.1 | ..... | ..... | ..... | ..... | ..... |
| 6.3 | 1.6 | 1.6 | ..... | ..... | ..... |
| 9.8 | 2.4 | 2.8 | 2.6 | ..... | ..... |
| 16.0 | 2.8 | 3.8 | 3.0 | 2.4 | ..... |
| 20.0 | 3.0 | 4.8 | 7.2 | 7.2 | 2.2 |
| 30.0 | 3.0 | 5.0 | *9.2 | 7.8 | 3.4 |
| 50.0 | 3.0 | *5.4 | 9.0 | 11.0 | 3.8 |
| 75.0 | 2.4 | 4.0 | 6.6 | 8.0 | 4.4 |
| 90.0 | 2.2 | 4.6 | 8.4 | 10.6 | 4.0 |
| 95.0 | 2.6 | 4.8 | 8.4 | *12.2 | *9.2 |
| 98.3 | 2.0 | 3.6 | 6.8 | 10.0 | 6.8 |

* Highest growth value for each temperature.

different for the two processes. Each growth graph slopes sharply upward at the beginning, quickly attaining the first or subordinate graph maximum. Each graph then slopes downward to the second or subordinate graph minimum and then again upward to the second or main graph maximum, beyond which it slopes sharply downward to its end.

The presence of two graph maxima with an intervening subordinate minimum renders these graphs of shoot elongation peculiarly interesting and makes it necessary to consider (as in the case of the corresponding graphs of $CO_2$ production) two optimal oxygen pressures for each temperature. The remarkable similarity in form of all the growth graphs makes it seem probable that this feature is highly significant and important. (It is to be borne in mind, however, that the data of shoot elongation were secured by a much less precise technique than that by which the data of $CO_2$ production were secured.) These graphs all agree in showing the second or main maximum with an oxygen pressure of 95 per cent. (90–95 per cent. for

20°). The pressure of 98.3 per cent. was clearly supra-optimal for shoot elongation, as well as for $CO_2$ production, with every temperature. The first graph maximum for shoot elongation corresponds to a pressure range of 20–50 per cent. for 10°; to a pressure of 50 per cent. for 15° and 25°; to a pressure of 30 per cent. (or perhaps a pressure range of 30–50 per cent.) for 20°; and for 30° this maximum is suggested for a pressure of 75 per cent. The intervening second minimum is remarkably well and consistently shown by the graphs for 15°, 20° and 25°, on which it corresponds to a pressure of 75 per cent. It is indicated as corresponding to a pressure of 90 per cent. on the graphs for 10° and 30°, however, temperatures that are respectively below and above the temperature most favorable to growth. The 30-degree graph remains exceptionally low for all percentages of oxygen until the abscissa for 90 per cent. is passed, when it ascends sharply to its maximum, for 95 per cent.

As in the case of $CO_2$ production, no reference to a double optimum of oxygen pressure for growth seems to have appeared in the literature and this feature of growth control in seedlings such as were used in this study is worthy of special attention, notably in connection with the corresponding double optimum of oxygen pressure for $CO_2$ production and all that is implied by such occurrences.

### Some comparisons between $CO_2$ production and shoot elongation as these were related to temperature and oxygen pressure

Possible competition between $CO_2$ production and shoot elongation.—In figure 7 it is seen that the pressure corresponding to the first or subordinate maximum on the graphs of $CO_2$ production for the whole experiment period is apparently the same as the highest oxygen pressure for which the corresponding growth graph has zero as ordinate. For the several temperatures these critical pressures are as follows: 10° and 15°, 3.1 per cent.; 20°, 6.3 per cent.; 25°, 9.8 per cent.; 30°, 16 per cent. $CO_2$ production was regularly more rapid with higher oxygen pressures up to the pressure that just allowed measurable shoot elongation, beyond which $CO_2$ production was notably lower with still higher pressures until the subordinate minimal region of the $CO_2$ graphs is passed. Although a considerable rate of $CO_2$ production may be supposed to be necessary for growth in such seedlings as these, yet the occurrence of growth may retard $CO_2$ production, as might be true if the two processes were competing, as it were, for the same plastic materials. The possibility that such competition may exist between growth and respiration were suggested by Copeland (9) who said that "the more active the respiration, the less the relative amount of plastic material left available for growth." That the minimal oxygen pressure required for growth is greater with progres-

sively higher temperatures within the range considered, is in agreement with the results of CANNON'S (7) experiments on the oxygen requirement of roots, and it appears that the higher temperatures may promote respiration more than they promote growth, other conditions being suitable. Of course the rate of $CO_2$ production should tend to increase with the growth rate when the latter is relatively great, for respiration must be supposed to proceed more rapidly as the number of active cells (or the volume of active protoplasm) increases with development and this may perhaps explain the second maxima in these curves. The highest percentage of oxygen tested generally gave somewhat lower rates of both shoot elongation and $CO_2$ production than did a slightly lower percentage with the same temperature. But neither growth nor $CO_2$ production approached zero rate with any tested temperature combined with the highest oxygen pressure tested, 98.3 per cent.

From the graphs in figure 7 it is apparent that the efficiency of respiration with respect to the amount of growth produced became greater as the oxygen pressure was increased above the main oxygen pressure minimum for growth, until the subordinate minimum of oxygen pressure for $CO_2$ production was passed. Above this region in the graph the efficiency of respiration gradually decreased until the subordinate minimum of oxygen pressure for growth was reached. For temperatures near the optimum for growth, the maximum efficiency of respiration was reached at oxygen pressures near that in ordinary air.

KOSTYTSCHEW points out (**25**, p. 54) that $CO_2$ production may not be greatly reduced by low partial pressures of oxygen in the surroundings, though the energy yield from respiration under such circumstances must be much less than when the oxygen pressure is higher. A rise in temperature may increase the respiration rate to a greater degree than it increases either the growth rate or the rate of transformation of plastic substances to available forms. The rate of growth may thus be limited on the one hand by the supply of available energy and on the other hand by the supply of plastic substances not used up in the respiration process.

TEMPERATURE INFLUENCE COMPARED WITH OXYGEN INFLUENCE WITH RESPECT TO $CO_2$ PRODUCTION AND SHOOT ELONGATION, BY MEANS OF COEFFICIENTS OF EFFECTIVENESS.—An idea of the relative influences of temperature and oxygen pressure on the rates of $CO_2$ production and shoot elongation, as shown by the results of these experiments, may be obtained by selecting an environmental complex that gave low rates and then ascertaining how much greater the rates were when one or the other or both of these two experimental variables were altered so as to give the greatest effect. What may be called coefficients of effectiveness (LIVINGSTON and SHREVE (**29**, p. 208) may be secured in this way for alterations of one or

more environmental conditions. We may take as our unit for shoot elongation the value 3.0 mm., which corresponds, let us say, to the temperature-oxygen combination of 10° and 20 per cent. (table V). With the temtemperature increased from 10° to 25° (the oxygen concentration being unchanged) the elongation rate was 7.2 mm., which is about 2.4 times our unit. On the other hand, when the temperature was not altered but the oxygen pressure was increased from 20 per cent. to 95 per cent. the rate value was only 2.6, which is lower than our unit, and the effectiveness of this oxygen-pressure change is consequently negative, its coefficient being 0.9. When both changes occurred together the rate was actually changed from 3.0 to 12.2 mm. and the coefficient of effectiveness of the double change is 4.7, which is 2.14 times as great as the calculated value, 2.2 (2.4 x 0.9). If the two environmental changes applied together had operated just as they did when applied separately, without mutual influence of antagonism or synergism (*i.e.*, if the effects had been simply additive), then the rate corresponding to the double change should have been 2.2 times as great as our unit. That the data actually show the coefficient of effectiveness of the double change as 4.7 instead of 2.2 indicates that when both changes were applied together the influence of one or both was greater than when they were applied separately. The two influences were synergistic in their action and the synergism resulted in an increase of effectiveness from 2.2 to 4.7.

For $CO_2$ production (table III) these same changes give 3.5 and 1.5 as the coefficients of effectiveness of the temperature alteration and of the oxygen-pressure alteration, respectively, while the coefficient of the double change is 8.3, which is 1.57 times the product of 3.5 and 1.5. These considerations show that the change from the temperature-oxygen combination of 10° and 20 per cent. to the combination of 25° and 95 per cent. was relatively much more effective to give more rapid $CO_2$ production than it was to give more rapid shoot elongation. Also it is indicated that, for both processes, the temperature change was much more important in this double alteration than was the oxygen change and that there was a pronounced synergistic action on both shoot elongation and $CO_2$ production, this being nearly twice as great for $CO_2$ production as for shoot elongation.

Such considerations as these may be valuable for comparisons when further experimental data become available on the effectiveness of temperature and oxygen pressure in controlling the rates of $CO_2$ production and of growth in organisms. For the present we may simply call attention to the possibilities of this method of analysis and give emphasis to the conclusions just presented.

## The cardinal values of temperature and oxygen pressure for $CO_2$ production and shoot elongation in the entire experiment period

Four sets of values considered.—The relations of temperature and oxygen pressure to $CO_2$ production and shoot elongation may be studied by comparing the two sets of cardinal values for the two experimental variables. The values to be considered are (1) the main minimal, optimal and maximal temperature (A) for $CO_2$ production and (B) for shoot elongation, and (2) the same critical value of oxygen pressure. From the data of tables III and V (figure 7) the three cardinal values of temperature for each oxygen pressure and the three cardinal values of each oxygen pressure for each temperature are first to be ascertained as nearly as possible.

Cardinal values for $CO_2$ production.—The minimal temperature for $CO_2$ production in the entire period was evidently below 10° for every oxygen pressure tested, presumably for every oxygen pressure below that of pure oxygen under 1 atmosphere of gas pressure. The maximal temperature for $CO_2$ production in the whole period was evidently above 30° for all oxygen pressures, but 30° is surely somewhat supra-optimal for health in seedlings like these and it is probably safe to suppose that that temperature was about the maximum for $CO_2$ production *in health*. With the behavior of the seedlings under injurious or lethal conditions we are not here concerned.

The optimal temperature for $CO_2$ production in the whole period was 30° or above for all oxygen pressures tested, but because 30° is to be considered as generally supra-optimal for health we may say that the optimal temperature for $CO_2$ production *in healthy seedlings* was, like the corresponding maximal temperature, probably not far from 30° for all oxygen pressures below 100 per cent.

No main minimal oxygen pressure for $CO_2$ production in the whole period is shown for any temperature. It is clearly shown to have been at least below 0.6 per cent. in all cases, but it seems likely that there is no such thing as a minimal oxygen pressure for $CO_2$ production in such organisms as these, for $CO_2$ would probably be produced at a measureable rate in the complete absence of oxygen, at least with the higher temperatures tested.

No maximal oxygen pressure for $CO_2$ production in the whole period is shown and it is evident that we must consider this critical value as above 100 per cent. for all temperatures tested. It would of course be impossible to have an oxygen pressure above 100 per cent. without at the same time introducing a presumably serious alteration in the environmental background; namely, a total gas pressure of more than 1 atmosphere.

The highest rate of $CO_2$ production for each temperature is shown in black-face type in table III. Of course the oxygen pressure corresponding to the highest rate for any given temperature is the main optimal pressure for that temperature. This main optimum varied with temperature, being shown as 90 per cent. for 10° and 30° and as 95 per cent. for 15°, 20° and 25°. It is remarkable that this value is indicated as below that of pure oxygen for every temperature tested.

Special attention has already been given to the occurrence of a subordinate optimal and a subordinate minimal oxygen pressure for $CO_2$ production in the whole period, both with low values, which range from 6.3 and 9.8 per cent. (10° and 15°) to 16 and 20 per cent. (30°).

Cardinal values for shoot elongation.—The minimal temperature for measurable shoot elongation in the entire period is seen (table V) to have been somewhat below 10° for every oxygen pressure for which data are available. This agrees with the corresponding observation for $CO_2$ production. But it is suggested that this minimal temperature for shoot elongation was probably lower for median pressures (20–50 per cent.) than for high or low pressures.

The maximal temperatures for measurable shoot elongation in the whole period is shown as low for low oxygen pressures and higher for higher oxygen pressures, being indicated between 15° and 20° for an oxygen pressure of 6.3 per cent., between 20° and 25° for a pressure of 9.8 per cent., between 25° and 30° for a pressure of 16 per cent., somewhat above 30° for pressure from 20 to 98.3 per cent. and farthest above 30° for a pressure of 95 per cent. These relations are very different from those for $CO_2$ production, for which no definite maximal temperature is shown for any oxygen pressure, although the temperature maximum was actually above 30° in every case.

The optimal temperature for measurable shoot elongation in the whole period is, like the corresponding maximal temperature, low for low oxygen percentages and higher for higher percentages. This optimum is indicated between 10° and 15° for a pressure of 6.3 per cent., about 15° for pressures of 9.8 and 16 per cent., about 20° for pressures of 20 and 30 per cent., and about 25° for pressures of 50–98.3 per cent. The temperature of 30° was unquestionably markedly supra-optimal for shoot elongation in the entire period, for every oxygen pressure employed. In this respect also the temperature relations of growths were very different from those of $CO_2$ production, for which no definite optimal temperature is shown for any oxygen pressure, although it was surely above 30° in every case.

The main minimal oxygen pressure for shoot elongation in the whole period varied with temperature, being higher for higher temperatures than for lower ones. It is indicated between 3.1 and 6.3 per cent. for 10° and

15°, between 6.3 and 9.8 per cent. for 20°, between 9.8 and 16 per cent. for 25°, and between 12 and 20 per cent. for 30°. This statement differs greatly from the corresponding statement concerning $CO_2$ production, for which no minimal oxygen pressure is shown for any temperature.

No maximal oxygen pressure for shoot elongation in the whole period is shown for any temperature and that maximum must have been above 98.3 per cent., if such a maximum existed. This is in agreement with the corresponding statement concerning $CO_2$ production.

The main oxygen-pressure optimum for shoot elongation in the whole period is shown at about 30 per cent. for 10°, probably between 30 and 50 per cent. for 15° and 20°, between 50 and 95 per cent. for 25° and about 95 per cent. for 30°. The oxygen pressure of 98.3 per cent. was clearly supra-optimal with respect to shoot elongation for every temperature tested. For $CO_2$ production this main optimal oxygen pressure is shown as 90 per cent. with 10° and 30°, and about 95 per cent. with temperatures from 15° to 25°.

The occurrence of two optimal oxygen pressures (or double optimum) for shoot elongation in the whole period has been considered above, where it is pointed out that $CO_2$ production and shoot elongation were apparently specifically related, in that the oxygen-pressure for shoot elongation is shown as having for each temperature the same value as the corresponding first optimal pressure for $CO_2$ production.

Optimal combinations of temperature and oxygen pressure for $CO_2$ production and shoot elongation.—The optimal temperature-oxygen combination for $CO_2$ production in the entire period, is shown as the combination of 30° and the pressure of 90 per cent., while the corresponding optimal combination for shoot elongation is shown as the combination of 25° and the pressure of 95 per cent. This constitutes an additional point of difference between the temperature-oxygen relations of the two processes. While the combination best suited to shoot elongation (25° and 95 per cent.) gave 12.2 mm. as the highest index of shoot elongation, the average mean hourly rate of $CO_2$ production corresponding to this index of elongation is only 190.8 mg., but the optimal combination for $CO_2$ production (30° and 90 per cent.) gave a corresponding $CO_2$ production of 232.6 mg. and the total amount of shoot elongation for that same combination of temperature and oxygen pressure is only 4.0 mm. per seedling for the experiment period. If the indices of shoot elongation (12.2 mm.) and $CO_2$ production (190.8 mg.) are both taken as unity for the combination of 25° and 95 per cent. the relative values of these indices for the combination of 30° and 90 per cent. become 0.3 (shoot elongation) and 1.2 ($CO_2$ production).

It is interesting to remark that the highest rate of shoot elongation shown may perhaps represent the developmental capacity of these seedlings,

for the background conditions and the duration factor employed. They had the capacity to develop shoots about 12.2 mm. long by the end of the experiment period if they were subjected to the temperature-oxygen combination of 25° and the oxygen pressure of 95 per cent. throughout the period, this development being accompanied by the production of about 1.9 mg. of $CO_2$ per seedling. None of the environmental complexes tested allowed any higher rate of shoot development but higher rates of $CO_2$ production actually occurred with combinations of 30° and an oxygen pressure of from 75 to 98.3 per cent. The capacity of these seedlings with respect to $CO_2$ production in the given period, and without regard to pathological or lethal phenomena probably related to the supra-optimal temperature of 30°, is shown as 232.6 mg. for 100 seedlings (or about 2.3 mg. per seedling), for the combination of 30° and the oxygen pressure of 90 per cent.

These observations constitute rather definite physiological characterizations of the seedlings used in this study and they may be valuable for purposes of comparison when systematic data of the sort presented in this paper shall have become available for other plant forms. We may suppose that any seed or seedling possesses a definite development or growth capacity, or a definite capacity for the production of $CO_2$, etc., and that an optimal environmental complex will permit the organism to attain its capacity, while failure to attain capacity in the specified time period implies a sub-optimal complex of environmental conditions. Of course it is logically possible that the optimal complex of conditions may be a broad range of complexes, or that there may be several different complexes each of which might allow or promote capacity performance. For performance considerably below capacity many different complexes may be suitable, as has been illustrated by the isopleths for $CO_2$ production in the second interval (figure 5), but as performance approaches the capacity of the organism the range of suitable complexes should become narrower. An interesting diagram of isopleths for shoot elongation may be constructed from the data of table V, but lack of sufficient space and the rather low degree of precision that characterizes these data have led to the omission of the growth diagram here.

It is possible that these seedlings might have exhibited a still greater capacity to grow or to produce $CO_2$ if the complex of environmental background conditions had been different; for example, if some toxic or stimulating gas were present in the gas stream. The capacity rate here emphasized is to be taken as holding, as far as present knowledge goes, only for the background complex used in these tests.

### The cardinal values of temperature and oxygen pressure for $CO_2$ production in the several observation intervals

In table II the highest rate of $CO_2$ production for each temperature is shown in bold-face type and of course these values correspond to the main optimal oxygen pressure for the respective temperatures. A study of these optimal pressures, in connection with the other cardinal values of oxygen pressure and the cardinal values of temperature, as they are alike or different for the several consecutive observation intervals, brings out a number of interesting relations, some of which will be mentioned below.

No minimal or maximal temperature with respect to $CO_2$ production is shown with any oxygen pressure tested (0.6 to 98.3 per cent.) in any of the five intervals. For all intervals, as for the whole period, the minimal temperature is shown as at least below 10°, and the maximal temperature is shown as above 30°, for every oxygen pressure tested. No optimal temperature is shown for any interval, but this value may be taken in every case as not far from 30° for all oxygen pressures, if only healthy seedlings are considered. Otherwise it may be considerably above 30°.

As in the case of the entire period, no main minimal oxygen pressure is shown for any interval or temperature; but this value was surely below 0.6 per cent. in every case. (As has been noted, there is reason for supposing that there is no such thing as a minimal oxygen pressure for $CO_2$ production in seedlings such as those here used.) No main maximum oxygen pressure is shown for any temperature in any interval, but this value was clearly above 98.3 per cent. in every case. The main optimal oxygen pressure is shown as varying with temperature and to some extent with the developmental phase of the plantlets. Its value is 90 per cent. for the first three intervals and 95 per cent. for the last two, for all temperatures tested, excepting that it is 75 per cent. for 10° in the second and 3rd intervals, 90 per cent. for 10° in the fourth and fifth intervals, 95 per cent. for 15° in the third interval.

In general, the later intervals are well represented by the second, for which the data have been presented rather fully by isopleths and plane graphs. The occurrence of a double optimum of oxygen pressure for $CO_2$ production is generally indicated for all temperatures in all intervals, the exceptions being of inconsistent occurrence or confined to temperatures that gave very low rates of $CO_2$ production with all oxygen pressures.

## Summary and conclusion

Nature of the experimentation.—This paper presents the results of an experimental study on $CO_2$ production and shoot elongation in very young wheat seedlings subjected to 60 different maintained environmental

complexes representing a wide range of temperatures and of partial pressures of oxygen in the culture solution in which the seedlings were submerged. The seedlings used were all very nearly alike, having been produced from selected seeds by means of a standard treatment. Their stage of development is indicated partly by the statement that the coleoptile was protruding less than a millimeter but that no roots had yet appeared, and partly by the specifications of the standard germination procedure that was regularly followed. One hundred seedlings were regularly used in each experiment.

The environmental background, including all the influential external conditions excepting the experimental variables, was very nearly the same for all experiments. This background portion of the maintained environment is described in detail, the main features being that the hundred seedlings of an experiment were submerged in 250 cc. of a standard, weak nutrient solution continually stirred by the passage of gas bubbles during the experiment period and that light was excluded from the culture chambers. The culture solution was not renewed and it doubtless changed in various ways (dependent on the activities of the seedlings) throughout the experiment period, which regularly extended for 46 hours after the close of a 2-hour period of transfer and adjustment, the previous germination period having lasted for 42 hours after the placing of the dry seeds in the water of the germination flask. The experiment period was subdivided into five consecutive observation intervals (of 4, 6, 12, 12 and 12 hours), for each of which the average hourly rate of $CO_2$ production was ascertained; there are thus available from each experiment five measurements of the rate of $CO_2$ production, representing different stages of the development of the seedlings, which of course changed internally as development advanced.

The experimental variables were maintained with very little fluctuation throughout the period of experiment, but they differed from experiment to experiment in a definite manner. They were the conditions of temperature and oxygen pressure, in 60 different combinations. Twelve different oxygen pressures were tested with each of five different maintained temperatures: 10°, 15°, 20°, 25°, 30° C. Any oxygen pressure was maintained by allowing a previously prepared mixture of oxygen and nitrogen to bubble continuously (at the rate of 20 cc. a minute) through the 250 cc. of nutrient solution in the culture flask. The twelve different oxygen-nitrogen mixtures contained 0.6, 3.1, 6.3, 9.8, 16, 20, 30, 50, 75, 90, 95 or 98.3 per cent. of oxygen by volume. Commercial gases were used in the preparation of the gas mixtures. The total gas pressure in the space above the solution in the culture flask was very little above the pressure of the outside atmosphere, the excess amounting to not more than 2 or 3 cm. of a mercury column.

Five experiments, all with the same partial pressure of oxygen but differing as to temperature, were carried out simultaneously in each experiment series and each series was repeated at least once before the final average rates of $CO_2$ production were computed. Some special repetitions near the end of the study showed clearly that the stock of seed used did not deteriorate appreciably while the work was in progress.

The final averages represent the rates of $CO_2$ production for the respective intervals, expressed as milligrams per hour, for 100 seedlings. The total amount of $CO_2$ produced by each culture was ascertained for each observation interval in each experiment period by absorbing the $CO_2$ in a measured quantity of standard $Ba(OH)_2$ solution, in an apparatus with some new features, and titrating the excess $Ba(OH)_2$ with standard HCl solution at the end of the interval. Observations on growth were made also and a numerical index of shoot elongation was secured from measurements made at the end of the entire experiment period.

Progressive increase in $CO_2$ production with time.—The average rate of $CO_2$ production was generally greater for later observation intervals than for earlier ones in the same experiment. A number of exceptions to this general rule receive special attention. They are mainly confined to differences between rates for the first and second intervals and to combinations of temperature of 20° or below with oxygen percentages of 16 or below. Neglecting the exceptions, the increase in $CO_2$ production as time went on was generally slow for combinations of low temperature and low oxygen pressure, more rapid for combinations of low temperature and high oxygen pressure, and still more rapid for combinations of high temperature and high oxygen pressure. This acceleration in the rate of $CO_2$ production was most pronounced for the combination of 30° and an oxygen percentage of 90 or 95. To illustrate the increase in the rate of $CO_2$ production throughout the five intervals the following representative series of average hourly rates may be useful:

| Temperature-oxygen combination | 1st interval | 2nd interval | 3rd interval | 4th interval | 5th interval |
|---|---|---|---|---|---|
| 10°, 20 per cent. | 0.39 | 0.41 | 0.45 | 0.53 | 0.61 |
| 20°, 20 per cent. | 0.82 | 0.84 | 1.07 | 1.33 | 1.67 |
| 20°, 95 per cent. | 1.02 | 1.38 | 2.20 | 3.60 | 5.01 |
| 30°, 95 per cent. | 1.87 | 2.55 | 4.54 | 6.18 | 6.87 |

Special attention is given to the manner in which the rate of $CO_2$ production increased with the progress of the experiment when the seedlings were subjected to the temperature-oxygen combination of 20° and 20 per cent., a combination probably frequently encountered in nature. The average rates shown under these conditions for the five intervals are respectively: 0.82, 0.84, 1.07, 1.33 and 1.67 mg. and the acceleration is seen to be almost uniform. The rate is shown to have increased by about 0.025 mg. per hour during the 33-hour period between about the 9th hour and about the 42nd hour.

No minimal or maximal combination of temperature and oxygen pressure for $CO_2$ production is shown.—No culture failed to give measurable $CO_2$ production with any tested combination of temperature and oxygen pressure in any interval and consequently no minimal combination or maximal combination is shown for any interval or for the whole experiment period. The lowest rate for every interval is for the combination of 10° and an oxygen pressure of 0.6 per cent., which gave rates of 0.26, 0.27, 0.28 and 0.29 mg. per hour for 100 seedlings, for the second, third, fourth and fifth intervals, respectively. The combination of the highest temperature tested (30°) with the highest oxygen pressure tested (98.3 per cent.) gave high average rates in all intervals. These averages are 2.42, 4.07, 5.47 and 6.38 mg. per hour for 100 seedlings, for the second, third, fourth and fifth intervals, respectively.

Optimal combination for $CO_2$ production.—The optimal combination of temperature and oxygen pressure for $CO_2$ production in the several intervals is shown as the combination of 30° and 90 per cent. (first three intervals) or 95 per cent. (last two intervals). In no case did the optimal combination include the highest oxygen pressure tested (98.3 per cent.). The highest rates shown for the second, third, fourth and fifth intervals are, respectively, 2.88 mg. (for 30° and 90 per cent.), 4.65 mg. (for 30° and 90 per cent.), 6.18 mg. (for 30° and 95 per cent.) and 6.87 mg. (for 30° and 95 per cent.).

Range of possibilities well represented by temperatures and oxygen pressures employed.—The graphs and tables show that the 5-degree temperature intervals used in this study might have been narrower without the introduction of confusion due to unexplained variation among the different lots of seedlings, and some of the wider intervals of oxygen pressure might also have been reduced in width. On the whole, however, the intervals chosen for sampling the logical possibilities of combinations of temperature and oxygen pressure proved to be satisfactory for such a survey as this.

Isopleths for equal rates of $CO_2$ production in the second interval.—The temperature-oxygen relations of $CO_2$ production in the second observation interval are set forth by a system of isopleths in a solid diagram and also by a set of plane graphs. The diagram shows clearly how the same rate of this process may result from many different combinations of temperature and oxygen pressure. For the region of this diagram between the lines for oxygen pressures of about 20 and about 90 per cent. the relation between temperature and oxygen pressure is shown to have been approximately linear for any rate value and the proportionality is about the same for all rates above 1.0 mg. Within this range the rate of $CO_2$ production for any oxygen pressure is shown to have been generally higher with higher temperature and the rate for any temperature was generally higher with higher oxygen pressure.

Double optimal oxygen pressures for $CO_2$ production.—For the several intervals and also for the entire period two optimal oxygen pressures for $CO_2$ production are generally shown with each temperature, these being the abscissae for two distinct graph maxima. Between the two graph maxima there is of course a subordinate graph minimum. The first or subordinate optimal oxygen pressure and the pressure corresponding to the second or subordinate graph minimum are generally higher with higher temperatures; they generally vary from about 6.3 per cent. and 9.8 per cent. (for 10°) to about 16 per cent. and 20 per cent. (for 30°). The second or main optimal oxygen pressure is generally shown as 90 or 95 per cent. The physiological meaning of the double optimum of oxygen pressure for $CO_2$ production, a feature apparently not previously mentioned in the literature of plant respiration and one that seems to be important, may be illustrated by a concrete example from the data for 25° and for the whole experiment period, as follows: The oxygen pressure of 9.8 per cent. (first or subordinate optimum for 25°) gave a much higher rate of $CO_2$ production than was given either by 6.3 per cent. or by 20 per cent., while the rates for 6.3 and 20 per cent. were about alike. And a much higher rate was given by 95 per cent. (main optimum for 25°) than by 9.8 per cent. The totals for these critical pressures, for 25° and for the entire period, are shown below.

| | |
|---|---|
| Lowest oxygen pressure tested (0.6 per cent.) | 46.0 mg. |
| First or subordinate optimal pressure (9.8 per cent.) | 98.4 mg. |
| Second or subordinate graph minimum (20 per cent.) | 80.4 mg. |
| Second or main optimal pressure (95 per cent.) | 190.8 mg. |

Neither for any of the observation intervals nor for the whole period did the highest oxygen pressure tested (98.3 per cent.) give the highest rate

of $CO_2$ production for any temperature, and the pressure 98.3 per cent. was consequently supra-optimal for $CO_2$ production in every instance.

Proportionality of $CO_2$ production to oxygen pressure.—As in the case of the second and later intervals, $CO_2$ production in the whole period is shown for any temperature as about proportional to oxygen pressure within the pressure range from that for the subordinate graph minimum to that for the main maximum; *i.e.*, between about 20 or 30 per cent., and about 90 or 95 per cent. The constant of proportionality is shown to be greater with higher temperature.

Cardinal temperatures for shoot elongation.—The data for shoot elongation in the entire experiment period are discussed in detail and their relations are compared to the corresponding relations of $CO_2$ production. No main minimal temperature is indicated for shoot elongation; it was clearly below 10° for every oxygen pressure tested. On the other hand, a minimal oxygen pressure for measurable shoot elongation in the experiment period is shown for every temperature tested and this critical pressure was progressively greater with higher temperature. The lowest pressures giving measurable shoot elongation were: for 10° and 15°, 6.3 per cent.; for 20°, 9.8 per cent.; for 25°, 15 per cent.; for 30°, 20 per cent.

Double optimal oxygen pressures for shoot elongation.—The graph for shoot elongation with each temperature generally shows three low or minimal regions and two high or maximal regions, when oxygen pressures are abscissae and indices of shoot elongation are ordinates. It is therefore necessary to consider a double optimum of oxygen pressure for each temperature, as in the case of $CO_2$ production, but the details are very different for the two processes. Such a double optimum of oxygen pressure seems not to have been recorded in the literature of plant growth and it appears to be important.

The first graph maximum for shoot elongation corresponds to a pressure range (first or lower optimal pressure) of 20–50 per cent. for 10°; to a pressure of 50 per cent. for 15° and 25°, to a pressure range of 30–50 per cent. for 20° and to a pressure of perhaps 75 per cent. for 30°. The second or main graph maximum corresponds to a pressure (second, upper, or main optimal pressure) of 90 or 95 per cent. for every temperature. The intervening second or subordinate graph minimum corresponds to a pressure of 75 per cent. with temperature of 15°, 20° and 25° and to a pressure of 90 per cent. with 10° and 30°.

It is remarkable that, for every temperature tested, the first or main minimal oxygen pressure for shoot elongation is shown as coincident with

the corresponding first optimal pressure for $CO_2$ production. $CO_2$ production in the whole period was regularly more rapid with higher oxygen pressure up to the pressure that just allowed measurable shoot elongation, beyond which $CO_2$ production was notably lower with still higher oxygen pressures until the subordinate minimal region of the $CO_2$ graph is passed. Although a considerable rate of $CO_2$ production may be supposed to have been necessary for growth, yet the occurrence of growth may have retarded $CO_2$ production, as might be true if the two processes were competing for the same plastic materials.

The highest oxygen pressure tested (98.3 per cent.) was supra-optimal for shoot elongation, as well as for $CO_2$ production, with every temperature employed.

Effectiveness of temperature and oxygen pressure in determining $CO_2$ production and shoot elongation.—The relative magnitudes of the temperature influence and the oxygen influence on the rate of $CO_2$ production and shoot elongation in the whole experiment period are examined by means of coefficients of effectiveness for environmental differences. Within the range of temperature-oxygen combinations studied, the temperature influence was much greater than the oxygen influence with respect to both processes, and both influences were more pronounced with respect to $CO_2$ production than with respect to shoot elongation. The two influences were generally synergistic when operating together; *i.e.*, the effect of a temperature difference and a simultaneous oxygen-pressure difference was greater than the summation of the effects of the two differences considered separately.

Cardinal temperatures for $CO_2$ production and shoot elongation compared.—The main cardinal values (minimum, maximum and optimum of temperature and of oxygen pressure) for $CO_2$ production and for shoot elongation in the whole experiment period, are studied and the two processes are compared with respect to these values. The minimal temperature for both processes was below 10° for every oxygen pressure for which data are available. The maximal temperature for $CO_2$ production in the whole period is shown as above 30° for every oxygen pressure tested, while for measurable shoot elongation it varied directly with the oxygen pressure; this maximum is shown as between 15° and 20° for an oxygen pressure of 6.3 per cent., between 20° and 25° for a pressure of 16 per cent., somewhat above 30° for pressures of 20–98.3 per cent. and farthest above 30° for a pressure of 95 per cent. The optimal temperature for $CO_2$ production was actually above 30° for every oxygen pressure tested but for shoot elongation this optimum varied directly with the oxygen pressure in the lower portion

of the pressure range. It is shown as 10°–15° for a pressure of 6.3 per cent., 15° for pressures of 9.8 and 16 per cent., 20° for pressures of 20 and 30 per cent. and 25° for pressures of 50–98.3 per cent.

Main cardinal oxygen pressures for $CO_2$ production and shoot elongation compared.—There is probably no such thing as a main minimal oxygen pressure for $CO_2$ production with any temperature, but for measurable shoot elongation in the experiment period the main minimal oxygen pressure is shown to have varied with temperature, being higher with higher temperature, as has been mentioned. The main maximal oxygen pressure for each temperature is shown as above 98.3 for both processes and attention is called to the consideration that an oxygen pressure higher than 100 per cent. would be impossible of attainment unless the total gas pressure were above 1 atmosphere. With respect to the main optimal oxygen pressures, as has been noted, the two processes differed very much. For $CO_2$ production this main oxygen pressure optimum was 90 per cent. with 10° and 30° and 95 per cent. with 15°, 20° and 25°, but it varied with temperature in quite another manner for shoot elongation.

The optimal combination of temperature and oxygen pressure for $CO_2$ production in the whole period is shown as the combination of 30° and 90 per cent. while the corresponding optimal combination for shoot elongation is shown as the combination of 25° and 95 per cent.

It is remarked that the highest rates of $CO_2$ production for the entire period and the corresponding total shoot elongation probably approximate the capacity of these seedlings for the given period. Thus, they had the capacity to develop shoots 12.2 mm. long by the end of the period if subjected to the optimal combination of temperature and oxygen pressure for this process, and they had the capacity to produce 232.6 mg. for 100 seedlings by the end of the period if subjected to the optimal combination for $CO_2$ production. These are physiological characterizations of the seedlings used in this study.

Cardinal temperatures and oxygen pressures for $CO_2$ production in the several intervals.—With regard to the cardinal values of temperature and oxygen pressure for $CO_2$ production in the several observation intervals, no minimal or maximal temperature is shown for any oxygen pressure in any interval. For all intervals, as well as for the whole period, these critical values are shown as respectively below 10° and above 30° for every oxygen pressure tested. As in the entire experiment period, no optimal temperature is shown for any oxygen pressure in any interval, and this value is indicated as above 30° in all instances, but that temperature was surely supra-optimal for health. No main minimal oxygen pressure is shown for

any temperature in any interval but this value was surely below 0.6 per cent. in every case, which is also true of the whole period. As has been noted, it is probable that there is no such thing as a main minimal oxygen pressure for $CO_2$ production with any temperature for seedlings such as these in any phase of their development. No main oxygen-pressure maximum for $CO_2$ production is shown for any temperature in any interval, but this was clearly above 98.3 per cent. in every case, just as for the entire period. On the other hand, the main optimal oxygen pressure for $CO_2$ production varied not only with temperature (as shown for the whole period) but also to some extent with the developmental phase of the plantlets. This main pressure optimum is generally shown as 90 per cent. for the first three intervals, and 95 per cent. for the last two intervals for all temperatures tested.

A FUNDAMENTAL PRINCIPLE EMPHASIZED.—The results of this whole study illustrate the most fundamental consideration for all research on the natural determination of process rates; namely, that the relation of any process to any influential condition or influence cannot be definitely stated without quantitative reference to the other prevailing influences. This is of course because of the inevitable concomitancy and interaction of all the influential conditions of the current environmental complex, including the time or duration factor. This general principle, which is basic for all physiological and ecological analysis, has been emphasized by BLACKMAN (**4**) in the following words: "The way of those who set out to evaluate exactly the effects of changes in a single factor upon a multi-conditioned metabolic process is hard, and especially so when this process is being pushed towards the upper limits of its activity."

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## VITA

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